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STRUCTURAL AND FUNCTIONAL ASPECTS OF CELL MEMBRANES

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. UBIQUITOUS CELL-SURFACE GLYCOPROTEIN ON TUMOR CELLS IS PROLIFERATION-ASSOCIATED RECEPTOR FOR TRANSFERRIN.

Sutherland R, Delia D, Schneider C, Newman R, Kemshead J, Greaves M

Membrane Immunology Lab., Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England

Proc Natl Acad Sci USA; 78(7,part2):4515-4519 1981

A murine monoclonal antibody (OKT9) raised against human leukemic cells binds to a wide variety of leukemia and tumor cell lines and to a minority of leukemia cells taken directly from patients. Fetal thymus and liver are strongly reactive as are some normal, immature hemopoietic cells and activated lymphocytes. Reactivity with OKT9 appears to correlate with proliferation status in both normal and malignant populations. Biochemical analysis indicates that this structure is a approx 180,000-dalton glycoprotein with two disulfide-bonded subunits of approx 90,000-daltons. Isolation of the transferrin receptor from a T-cell line (MOLT-4) indicates that it also has

HE 20.3173/2:

a dimeric approx 180,000-dalton structure. Radio-labeled transferrin bound to its receptors can be specifically precipitated by the monoclonal OKT9, although the latter does not bind transferrin itself, indicating that the antigenic structure defined by this antibody is likely to be the transferrin receptor. (Author abstract) (37 Refs)

2. ECTO-ADENOSINE TRIPHOSPHATASE DEFICIENCY IN CULTURED HUMAN T AND NULL LEUKEMIC LYMPHOCYTES. A BIOCHEMICAL BASIS FOR THYMIDINE SENSITIVITY.

Fox RM, Piddington SK, Tripp EH, Tattersall MH
Ludwig Inst. Cancer Res., Univ. Sydney, New South Wales 2006, Australia
J Clin Invest; 68(2):544-552 1981

Cultured leukemic T and null lymphocytes are highly sensitive to growth inhibition by thymidine, as well as the other deoxynucleosides, deoxyguanosine and deoxyadenosine. By contrast, Epstein-Barr virus-transformed B lymphocytes are relatively resistant to deoxynucleosides. Growth inhibition is associated with the development of high deoxyribotriphosphate pools after exposure to the respective deoxynucleosides. We show that malignant T and null lymphocytes are deficient in ecto-ATPase activity. We show this cell surface enzyme to be of broad specificity, capable of degrading both ribotriphosphates and deoxyribotriphosphates. High levels of this ecto-enzyme are found in deoxynucleoside-resistant, Epstein-Barr virus-transformed B lymphocytes. Ecto-ATPase deficiency may represent a mechanism for increased sensitivity to deoxynucleoside growth inhibition. (Author abstract) (28 Refs)

3. STRUCTURAL PROPERTIES AND TISSUE DISTRIBUTION OF THE ANTIGEN RECOGNIZED BY THE MONOCLONAL ANTIBODY 653.40S TO HUMAN MELANOMA CELLS.

Natali PG, Imai K, Wilson BS, Bigotti A, Cavaliere R, Pellegrino MA, Ferrone S
(c/o Ferrone) Dept. Molecular Immunology, Scripps Clinic and Res. Foundation, La Jolla, CA, 92037
JNCI; 67(3):591-601 1981

The hybridoma 653.40S, constructed with splenocytes from an inbred BALB/c mouse immunized with cultured human melanoma cells, secreted an antibody that had been shown to recognize an antigenic determinant restricted to human melanoma cell lines. The monoclonal antibody (MoAb) 653.40S showed immunoprecipitation of two glycopolypeptides synthesized by melanoma cells, one with the apparent mol wt of 280,000 and the other one with a mol wt larger than 500,000. These two glycopolypeptides were not bridged by disulfide bonds and were peripheral rather than integral to the plasma cell membrane. Comparison of the reactivity of cells of the melanocyte lineage with the MoAb 653.40S and with the MoAb Q5/13 to human Ia-like antigens showed that the former reacted with proliferating melanocytes and melanoma cells, whereas the latter reacted only with melanoma cells. The MoAb 653.40S did not react with a large variety of surgicall y removed normal and tumor tissues except for some instances of basal cell and squamous cell carcinomas. These results suggested that double staining of pigmented skin lesions with the MoAb 653.40S and with an MoAb to Ia-like antigens may help to solve controversial diagnosis of melanoma. Furthermore, the MoAb 653.40S may be useful for radioimaging and immunotherapy of melanoma. (Author abstract) (32 Refs)

4. STRUCTURAL CHARACTERIZATION OF HUMAN MELANOMA-ASSOCIATED ANTIGEN P97 WITH MONOCLONAL ANTIBODIES.

Brown JP, Nishiyama K, Hellstrom I, Hellstrom KE
Div. Tumor Immunology, Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
J Immunol; 127(2):539-546 1981

Monoclonal antibodies were used to study p97, a human melanoma-associated antigen (MAA). Four hybridomas, designated 4.1, 96.5, 118.1, and 8.2, were obtained by fusing mouse myeloma cells with spleen cells from mice immunized with human melanoma cells. Antibodies 4.1 and 8.2 were IgG1; antibodies 96.5 and 118.1 were IgG2a. Sequential immunoprecipitation (IP) and sodium-dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that all four antibodies recognized the same 97 kilodalton (kD) protein. Binding studies with 125I-labeled antibody showed that antibodies 4.1 and 96.5 bound the same epitope, p97a. Antibodies 118.1 and 8.2 defined epitopes p97b and p97c, respectively. Six monoclonal antibodies (M17, L1, L10, R10, I12, and K5) specific for gp95, a 95 kD melanoma cell surface glycoprotein were also tested. Sequential IP showed that these antibodies bound p97; p97 and gp95 are thus identical. Binding studies showed that antibody M17 bound epitope p97a, and antibodies L1, L10, and R19 bound epitope 97c. Antibodies I12 and K5 defined two other epitopes, p97d and p97e, respectively. SDS-PAGE under on reducing conditions indicated that p97 is monomeric, probably with intrachain disulfide bonds. Cell-surface labeling of sialic acid residues and neuraminidase digestion showed that p97 is a sialoglycoprotein. Digestion of p97 with papain or trypsin produced a stable 40 kD fragment, which expressed epitopes p97a, p97b, and p97c, but not p97d or p97e. (Author abstract) (35 Refs)

5. CATHEPSIN B-LIKE ENZYMES. SUBCELLULAR DISTRIBUTION AND PROPERTIES IN NEOPLASTIC AND CONTROL CELLS FROM HUMAN ECTOCERVIX.

Pietras RJ, Roberts JA
Dept. Biology, Univ. California, Los Angeles, CA, 90024
J Biol Chem; 256(16):8536-8544 1981

Distribution of cathepsin B-like activity was determined in fractions of control and neoplastic epithelial cells from human ectocervix. Neoplastic cells with a moderate rate of proliferation (NCE-1) were separated from those in the same primary tumor with higher replication rates (NCE-2). In each cell line, activities of acid phosphatase and beta-glucuronidase were concentrated in mitochondria-lysoso me (ML) fractions. Cathepsin activity was also concentrated in ML fractions of control but not in those of neoplastic cells, with activity largely in crude nuclear (CN) fractions of NCE-2. ***Plasma membranes*** (PM) with densities of 1.13-1.16 g/ml were purified from CN fractions. The molar ratio of cholesterol to phospholipid was 0.91 in PM of controls and 0.88 to 0.89 in PM of cancer cells. Activities of plasmalemma marker enzymes in PM were enriched by 30 to 40x homogenate levels and specific binding sites for wheat germ agglutinin were enriched by 34-to 44-fold. In neoplastic, but not control, cells, cathepsin activity was also enriched in PM to as high as 14x homogenate levels in NCE-2. This activity was reduced no more than 6% by extraction of membranes with hypotonic or high salt buffers. Enzyme in fractions of tumor and control cells cleaved the endopeptidase substrate, benzyloxycarbonyl-Ala-Arg-Arg-4-methoxy-beta naphthylamide, at a pH optimum of 6.2 at 37 C, with a Km of 0.038 mM in ML of controls and 0.056 to 0.058 mM in PM and purified nuclei of NCE-2. Activities were inhibited strongly by

iodoacetic acid, ZnSO4, leupeptin, antipain, and tosyl-L-lysine chloromethyl ketone, whereas pepstatin, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride EDTA, and CaCl2 were less effectove. Cathepsin B-like enzyme in ML fractions of control and cancer cells was unstable at pH 8.0 and sensitive to heat denaturation at 65 C. In contrast, activity in PM and nuclei of NCE-2 cells showed considerable stability at pH 8.0 and 65 C. Thus, heat- and alkaline-stable cathepsin B-like activity occurs in plasma membranes and nuclei of neoplastic cervical cells. (80 Refs)

6. BREAST EPITHELIAL CELL SURFACE COMPONENTS CHARACTERIZED BY MONOCLONAL ANTIBODIES (MEETING ABSTRACT).

Peterson JA, Ceriani RL
Bruce Lyon Memorial Research Laboratory, Children's Hospital Medical Center, Oakland, CA, 94609
4th Annual San Antonio Breast Cancer Symposium, Nov 6-7, 1981, San Antonio, Texas Cancer Therapy and Research Center of South Texas; 1981

Monoclonal antibodies were prepared to components of the cell surface of breast epithelial cells using human milk fat globule membranes (HMFGM) as a source of antigens. Spleen cells from BALB/c mice, immunized with HMFGM, were fused with either producing or non-producing mouse myeloma cells. The resulting hybridomas were screened by a solid phase radioimmunoassay against membranes of normal breast epithelial cells and a battery of non-breast cells, and only those with a high or complete specificity for breast epithelial cells were retained. With a solid phase methodology it was possible to determine mol wt, and so far several monoclonal antibodies have been prepared that detect cell surface components with apparent mol wt of 45,000 and 400,000 respectively. The later component was a glycoprotein with a very high sugar cont ent. Further, quantitative levels of these antigens in normal and neoplastic breast epithelial cells were obtained by a competitive radioimmunoassay. Both by radioimmunoassay and by indirect immunofluorescence it was established that human breast tumors are composed of heterogeneous cell populations where the antigens detected by our battery of monoclonals vary quantitatively from cell to cell, and are sometimes even deleted. However, each cell line or breast tumor has a fixed pattern of expression of these antigens. This variability of breast tumor cells contrasts with rigid expression of normalcy found in normal breast tissue. (no Refs)

7. SYNTHESIS OF ALPHA-1-ACID GLYCOPROTEIN AND ALPHA-1-ANTICHYMOTRYPSIN BY BENIGN AND MALIGNANT HUMAN BREAST EPITHELIAL CELLS (MEETING ABSTRACT).

Gendler SJ, Tokes ZA
Department of Biochemistry and the Comprehensive Cancer Center, University of Southern California, School of Medicine, Los Angeles, CA, 90033
4th Annual San Antonio Breast Cancer Symposium, Nov 6-7, 1981, San Antonio, Texas Cancer Therapy and Research Center of South Texas; 1981

Human breast epithelial cells form well-differentiated, three-dimensional glandular structures. To form these structures, breast tissue undergoes dynamic reorganization which requires proteolytic remodeling and deposition of basal lamina. Glycoproteins have been implicated in these cell-cell interactions. Since glycoprotein expression is under control of the cells social interactions, glycoprotein synthesis and release were followed by incorporation of 14C-glucosamine in short term organ cultures of human breast surgical specimens which can be maintained with minimum alteration of 3-dimension (D) tissue integrity. We now present evidence that human breast epithelial cells actively synthesize alpha-1-antichymotrypsin (Achy) and alpha-1-acid glycoprotein (AGP). Analysis by 2-D gel electrophoresis and quantitative immunoprecipitation show that Achy accounts for 0.9% to 11.9% and AGP makes up 0.7% to 3.1% of the total of biosynthetically-labeled glycoproteins. To confirm the epithelial origin of these glycoproteins, the established cell lines MCF-7 and MDA-MB-231 were shown to synthesize and secrete them. Achy and AGP are detected at the cell surface as well. Monolayer cultures of these cells have 3.0% to 4.2% of Achy and 2.4% to 5% of AGP retained at the cell surface after extensive washing. Achy is a known inhibitor of serine proteinases with strong affinity for cathepsin G. AGP functions as a potent immunomodulator by suppressing lymphoblastogenesis, as well as by acting as a proteinase inhibitor. These glycoproteins may play regulatory roles in proteolytic processing of breast tissue and may act as the tissue's own protecting shield against invading WBC. (no Refs)

8. ENDOGENOUS PROTEIN PHOSPHORYLATION IN ISOLATED PLASMA MEMBRANES OF AH-66 HEPATOM A ASCITES CELLS.

Nakajo S, Nakaya K, Nakamura Y
Lab. Biological Chemistry, Sch. Pharmaceutical Sciences, Showa Univ., Hatanodai, Shinagawa-ku, Tokyo 142, Japan
J Biochem (Tokyo); 90(1):23-31 1981

The phosphorylation of endogenous membrane proteins by an endogenous protein kinase was studied in isolated plasma membranes from AH-66 hepatoma ascites cells using [gamma-32P]ATP as a precursor. The phosphorylation occurred very rapidly in the presence of 10 mM Mg++ and reached a max level at 2 min. Ca++ strongly inhibited the phosphorylation reaction and antagonized the activation produced by Mg++. Neither cyclic AMP nor cyclic GMP had a significant effect on the phosphorylation. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography showed that only membrane proteins ranging in mol wt from 125,000 to 200,000 were heavily phosphorylated and those of less than 60,000 mol wt were not phosphorylated at all. The protein kinase activity was readily extractable from the plasma membranes with 1 mM EDTA at pH 8.5. Among exogenous substrates, the extracted protein kinase catalyzed the phosphorylation of histone, protamine and phosvitin rather than casein. When the extracted protein kinase was subjected to chromatography on DEAE-Sepharose, a single major peak of cyclic AMP-independent protein kinase was eluted at a position quite different from those of the cytosolic protein kinases. (Author abstract) (23 Refs)

9. EGF AND CAMP DEPENDENT PROTEIN KINASE ACTIVITIES IN MURINE LEYDIG CELL TUMOR MEMBRANES (MEETING ABSTRACT).

Melner MH, Puett D
Department of Biochemistry, Vanderbilt University, Nashville, TN, 37232
63rd Annual Meeting of the Endocrine Society, June 17-19, 1981, Cincinnati, Ohio. The Endocrine Society 385 pp., 1981.

The present study was designed to investigate hormone-responsive protein kinase activity in the M5480P murine Leydig cell tumor. Plasma membranes were purified by double phase dextran-polyethylene glycol gradients and shown to contain two distinct protein kinase activities. One protein kinase activi-

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ty is stimulated by the cyclic nucleotides 3',5' cyclic (c)AMP and 3',5' cyclic guanosine monophosphate (cGMP). cAMP is more effective than cGMP with and ED50 of 3.1 uM versus 2.2 mM, respectively. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the membrane reactions, followed by autoradiography, revealed the phosphorylation of two relatively low mol wt proteins (aprox 12,000-18,000). Another protein kinase activity is detectable which is stimulated by mouse epidermal growth factor (EGF). SDS-PAGE of these membrane phosphorylation reactions demonstrates the selective phosphorylation of a protein of greater than 130,000 mol wt. The two kinase activities can be further distinguished by their susceptibility to modification by the transition elements chromate and molybdate. The presence of 10 mM sodium chromate in the reaction mixture increases the specific EGF-stimulated kinase activity 10-fold over controls. No apparent change is observed with the cAMP-dependent kinase activity under identical conditions. Sodium molybdate (10 mM) increases EGF-stimulated kinase activity approx 60% over controls while cAMP-dependent kinase activity is unaffected. In conclusion, purified plasma membranes from murine Leydig cell tumors exhibit two apparently distinct protein kinase activities stimulated by EGF and cAMP. (no Refs)

10. COMPARISON OF EHRLICH ASCITES TUMOUR AND MOUSE LIVER CELLS BY ANALYTICAL SUBCELLULAR FRACTIONATION COMBINED WITH A SENSITIVE COMPUTATIONAL METHOD FOR DATA ANALYSIS.

Nelson WJ, Nelson SJ, Traub P
Max-Planck-Institut fur Zellbiologie, Rosenhof, D-6802 Ladenburg, W. Germany
Hoppe Seylers Z Physiol Chem; 362(7):903-918 1981

A simple method of analytical subcellular fractionation, combined with a sensitive computational method for data analysis and presentation, has been used to reinvestigate the distribution and relative amounts of several enzymes in the cytoplasmic and plasma membranes of two different cell types: one is a neoplastic, transformed cell type (Ehrlich ascites tumor cells), the other an untransformed, highly differentiated cell type (liver hepatocytes plus Kupffer and endothelial cells). In general the distribution of the enzymes in particular membranes is similar in the two cell types, however the relative amounts differ. Ehrlich ascites tumor cells have a higher specific activity of galactosyltransferase and ouabain-sensitive (Na+, K+)ATPase, while liver cells have higher glucose-6-phosphatase, 5'-nucleotidase and succinate dehydrogenase activity. These differences appear to be correlated with morphological and, in some cases, functional differences between the two cell types. (Author abstract) (63 Refs)

11. HYALURONATE-PROTEIN COMPLEX OF ROUS SARCOMA VIRUS-TRANSFORMED CHICK EMBRYO FIBROBLASTS.

Mikuni-Takagaki Y, Toole BP
(c/o Toole) Dept. Anatomy and Cellular Biology, Tufts Univ. Sch. Medicine, Boston, MA, 02111
J Biol Chem; 256(16):8463-8469 1981

Involvement of covalently linked protein or peptide in the structure or synthesis of hyaluronate has not previously been convincingly demonstrated. We have developed conditions for double-labeling with [3H]leucine and [14C]acetate, then isolating and characterizing the cell-associated and secreted hyaluronate-protein complexes of Rous sarcoma virus-transformed chick embryo fibroblasts. The preparations were purified by Bio-Gel A-15m gel filtration and CsCl density gradient ultracentrifugation under dissociative conditions, followed by acid agarose gel electrophoresis in the presence of 0.1% Nonidet P-40. The purified hyaluronate preparations did not change their 3H:14C ratios after further sodium dodecyl sul fate or alkali treatment. The cell-derived hyaluronate-protein was resistant to pronase but susceptible to proteinase K in the presence of sodium dodecyl sulfate. After chondroitinase ABC digestion, the cell-derived 3H-labeled protein was separated from the 14C-labeled hyaluronate disaccharides, then shown to give a broad band corresponding to Mr approx = 12,000 on sodium dodecyl sulfate-urea polyacrylamide gel electrophoresis and to be susceptible to both pronase and proteinase K. The corresponding 3H-labeled peptide was prepared in the same manner from the medium hyaluronate and the [3H]leucine shown to be present in material smaller in amount and size than that from the cell. We propose from these and other published data that the cell-associated hyaluronate-protein may be bound to the cell surface and that the hyaluronate in the medium may be derived from it as a result of proteolytic scission. (Author abstract) (48 Refs)

12. CHANGES IN CELL SURFACE PROTEINS DURING DIFFERENTIATION OF MOUSE EMBRYONAL CARCINOMA CELLS.

Howe CC, Solter D
Wistar Inst. Anatomy and Biology, 36th St. at Spruce, Philadelphia, PA, 19104
Dev Biol; 84(1):239-243 1981

The cell surface proteins of teratocarcinoma-derived embryonal carcinoma cells (ECC), of parietal endoderm (Pys-2 and F9-AC cl 9), and of fibroblasts (OTT6050f) were radioiodinated by a lactoperoxidase method and analyzed by two-dimensional gel electrophoresis. The combined electrophoretic profiles from a number of ECC lines allowed the determination of eight ECC-unique polypeptides. Parietal endoderm and fibroblast expressed their own unique polypeptides. The two parietal endoderm-specific polypeptides are identical to the subunits of laminin. Retinoic acid-induced differentiation of one ECC line (F9) resulted in the disappearance of polypeptides specific for ECC and the appearance of those specific for the parietal endoderm. (Author abstract) (34 Refs)

13. LIPID PATTERNS OF EMBRYONAL CARCINOMA CELL LINES AND THEIR DERIVATIVES: CHANGES WITH DIFFERENTIATION.

Coulon-Morelec MJ, Buc-Caron MH
(c/o Buc-Caron) Dept. Pharmacology, Harvard Medical Sch., Boston, MA, 02115
Dev Biol; 83(2):278-290 1981

The lipid composition of several teratocarcinoma cell lines has been examined by biochemical and immunological methods in order to identify properties that might be correlated with the state of cell differentiation. The data indicate qualitative and quantitative changes in the phospholipid, cholesterol, and glycolipid composition. In particular, the ratios of cholesterol/phospholipid and of sphingomyelin/phosphatidylcholine are higher in differentiated cells. Gangliosides with short glycosidic chains (GM3 and GD3) are characteristic of undifferentiated, multipotent, embryonal carcinoma cell lines. More complex gangliosides (GM1 and GD1a) appear early during the course of diffe rentiation. Each differentiated cell line presents a unique ganglioside map. Results are tentatively correlated with a stabilization of the membrane bilayer in differentiated cell lines, whereas a more fluid state of the membrane in embryonal

carcinoma cell lines would allow max flexibility. Subtle differences in ganglioside 19 composition among embryonal carcinoma cell lines are discussed in relation with their potentialities, and their developmental age. (Author abstract) (40 Refs)

MORPHOLOGICAL STUDIES

14. DIFFERENCES IN THE MORPHOLOGY OF HERPES SIMPLEX VIRUS INFECTED CELLS. II. TYPE SPECIFIC MEMBRANE ALTERATIONS OF HSV-1 AND HSV-2 INFECTED CELLS.

Hampl H, Schlehofer JR, Habermehl KO
(c/o Schlehofer) Institut fur Virologie, Zentrum fur Hygiene, Albert-Ludwig-Univsersitat, Hermann-Herder-Strasse 11, D-7800 Freiburg, W. Germany
Med Microbiol Immunol (Berl); 169(3):209-223 1981

Morphological and functional differences produced by infection with Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) were studied in HEp-2 cells and in primar chick embryo fibroblasts (CEF). The four strains of HSV-1 (THEA, KOS, LS5039, and HFEM) tested, differed only slightly in their ability to induce a reduction of the membrane permeability in HEp-2 cells. The release, as well as the uptake, of 51Cr was reduced following HSV-1 infection. In addition, HSV-1 strains induced a stabilization of the membrane of HEp-2 cells towards the detergent Triton-X-100. In contrast, the membrane permeability of HEp-2 cells was enhanced by all strains of HSV-2 tested (DD, D316, and 196) and stabilization was not achieved. In CEF cells, HSV-1 and HSV-2 caused effects on the membrane permeability different from those in HEp-2 cells: enhancement of the 51Cr-release by HSV-1 (THEA: enhancement by 10.5% compared to mock-infected cells, LS5039: 34.1%, HFEM: 25.5%) vs reduction of release by HSV-2 (D316:-11.9%, DD:-11.3%). The type-specific functional alterations of the surface membrane were well correlated with structural differences shown by comparative electron microscopic investigation. Ultramicroscopic findings following infection with HSV-1 and HSV-2 strains are described. The results suggested that morphological and functional differences observed in infected cells depend, in part, on the type (HSV-1 or HSV-2) and strain of virus, and on the type of cell infected. (57 Refs)

15. ELECTRON MICROSCOPY OF POLYPS OF THE COLON: I. COMPARATIVE STUDY OF THE EPITHELIAL CELLS OF ADENOMATOUS AND ADENOPAPILLARY POLYPS.

Balazs M
Dept. Pathology, Janos Hosp., Diosarok u. 1, H - 1125 Budapest, Hungary
Exp Pathol (Jena); 19(2):112-121 1981

By comparing the electron-microscopic pictures of adenomatous and adenopapillary polyps, the ultrastructural characteristics of premalignant epithelial dysplasia were studied. In highly dif ferentiated adenomatous polyps, the ratio and the electron-microscopic appearance of absorptive and goblet cells did not differ from the epithelial cells of the normal mucosa of the colon. In the dysplastic regions of adenopapillary polyps, the intensive proliferation in several layers of undifferentiated epithelial cells, and the maturation disorder of absorptive and goblet cells, were observed. Severe changes occurred on the cell membrane as well as atypical vacuoles and dense bodies in the apical regions of the cell membrane. Several inclusion bodies of Leuchtenberger were observed, these being considered an important characteristic of malignant transformation. (Author abstract) (37 Refs)

16. ULTRACYTOCHEMICAL LOCALIZATION OF ALKALINE PHOSPHATASE ISOZYMES IN HUMAN CANCER CELL LINES IN VITRO.

Takeya M
Second Dept. Pathology, Kumamoto Univ. Sch. Medicine, Kumamoto 860, Japan
Acta Histochem Cytochem; 14(3):223-235 1981

The ultrastructural localization of isozymes of alkaline phosphatase (AP) was investigated in four human cancer cell lines: chorion isozyme in KMK-2 cells derived from human gastric carcinoma, Regan isozyme in HeLa S3-10 cells and CaR-1 cells derived from human rectal carcinoma, and Kasahara isozyme in HeLa S3-5 cells. The chorion isozyme in KMK-2 cells was shown to be almost exclusively on the microvilli facing the culture medium and not on the lateral surface adjacent to the neighboring cells with interdigitating folds. In both HeLa sublines, the Regan and Kasahara isozymes were found on the whole plasma membrane. The Regan isozyme in CaR-1 cells was strongly shown in the intracytoplasmic microcysts and in the whole plasma membrane. The cytochemical results suggested that all the isozymes were principally localized on the surface of the plasma membrane. It is suggested that a close relationship exists between primitive AP and its differentiation which may be controlled by intracellular genes. The AP in cancer cells have a tendency to develop to various degrees of differentiating stage and in relation to the organ-specific situation according to the regulation of altered genes. (29 Refs)

17. ULTRASTRUCTURAL PECULIARITIES OF INTERCELLULAR JUNCTIONS IN BRONCHOPULMONARY CARCINOMAS.

Nicolescu PG, Eskenasy A, Mangiulea V, Diaconita G, Stefan M
Dept. Pathology, Inst. Medicine and Pharmacy, Bucharest, Rumania
Morphol Embryol; 27(2):133-136 1981

The results of electron microscope examination of six specimens of bronchopulmonary carcinomas are reported. Histologically, there were two epidermoid spindle cell carcinomas, two poorly differentiated epidermoid carcinomas, and two large cell carcinomas. Characteristic ultrastructural features of poorly differentiated epidermoid and large cell anaplastic carcinomas were reduced number and irregular contour of intercellular junctions. Differentiated spindle cell carcinomas were characterized by the presence of desmosomes. (11 Refs)

18. ULTRASTRUCTURAL STUDY OF MEMBRANE GLYCOCALYX IN PRIMARY AND METASTATIC HUMAN AND RAT MAMMARY CARCINOMA.

Ghosh L, Nassauer J, Faiferman I, Ghosh BC
NCI, 7910 Woodmont Ave., Rm. 4804, Bethesda, MD, 20205
J Surg Oncol; 17(4):395-401 1981

The glycocalyx of primary and metastatic tumors was compared by ruthenium red staining and electron microscopy. Two systems were studied: human primary and metastatic breast tumors and rat primary and metastatic mammary carcinomas. A considerable diminution in the amount of glycocalyx was detected in both systems when primary tumors were compared with metastases. These results are consistent with observations of others showing that metastatic tumors shed glycoprotein antigens to the circulation; this may provide an immune escape

mechanism for tumor cells, allowing their dissemination. (Author abstract) (16 Refs)

MEMBRANE RECEPTOR/ BINDING SITES

19. INDUCTION OF COMPLEMENT RECEPTOR EXPRESSION IN CELL LINES DERIVED FROM HUMAN UNDIFFERENTIATED LYMPHOMAS. II. CHARACTERIZATION OF THE INDUCED COMPLEMENT RECEPTORS AND DEMONSTRATION OF THE SIMULTANEOUS INDUCTION OF EBV RECEPTOR.

Magrath I, Freeman C, Santaella M, Gadek J, Frank M, Spiegel R, Novikovs L

Pediatric Oncology Branch, NCI, Bethesda, MD, 20205

J Immunol; 127(3):1039-1043 1981

We have studied the specificity of complement receptors induced by theophylline in two cell lines derived from undifferentiated lymphomas, one of Burkitt's type, and compared it to that of complement receptors in other cell types. Both C3b and C3d receptors were induced. The induced C3b receptor differed from the C3b receptor of mature normal lymphocytes, polymorphonuclear leukocytes and the cells of a nodular lymphoma in two respects. Firstly, it bound C3b much less avidly (by a factor of several hundred-fold) and secondly, we were unable to demonstrate C4b binding. EBV receptors were induced at the same time as complement receptors, and permitted the conversion of a greater fraction of cells to EBNA positivity after experimental infection with EBV. The induction of receptors was not associated with a change in the fluidity of the plasma membranes and our data do not favor a different orientation of induced receptors within the membrane as compared to receptors of other cell types - a potential explanation for the different specificities. Our findings are consistent with the possibility that the complement receptors of lymphocyte precursors differ from those of mature lymphocytes. (Author abstract) (20 Refs)

20. ACUTE LYMPHOCYTIC LEUKEMIA AND COMPLEMENT RECEPTORS (A STUDY OF 45 CASES).

De Rossi G, Gugliemi C, Lopez M, Pasqualetti D, Mandelli F

Cattedra di Ematologia, Universita degli Studi di Roma, Via Arno 62, 00198 Roma, Italy

Ric Clin Lab; 11(2):139-143 1981

The relationship between the expression of complement receptors (C3R) and the expression of sheep erythrocyte receptors (ER) and surface membrane immunoglobulins (SmIg) was studied in 45 patients with acute lymphocytic leukemia (1-47 yr old) (ALL). Of 45 patients, 34 were at the onset of ALL, 9 were in the first relapse, and 2 were at onset and in the first relapse. C3R was constantly associated with SmIg (B cell ALL); C3R was frequently but not always associated with ER (T cell ALL). In three patients, C3R was not associated either with SmIg or with ER. (20 Refs)

21. CHARACTERIZATION OF SERUM-INDUCED ALTERATIONS IN THE CYCLIC AMP PATHWAY IN S49 LYMPHOMA CELLS.

Darfler FJ, Hughes RJ, Insel PA

Div. Pharmacology, Dept. Medicine, Univ. California, San Diego, La Jolla, CA, 92093

J Biol Chem; 256(16):8422-8428 1981

We have grown the S49 lymphoma cell line in a serum-free, chemically defined medium which contains casein, insulin, transferrin, testosterone, and linoleic acid. When grown in this medium, wild type S49 cells display a two- to three-fold increase in hormonal (beta-adrenergic, prostaglandin E1) and nonhormonal (cholera toxin) stimulation of cAMP accumulation compared to cells grown in 10% horse serum. Wild type cells grown in serum-free medium contained 30% more ***beta-adrenergic receptors*** than did serum-grown cells, although the affinity of the receptors for [3H]dihydroalprenolol was unchanged. The S49 variants, UNC and kin-, failed to show the alterations in cholera toxin- or isoproterenol-stimulated cAMP accumulation that were observed with wild-type cells. 125I-labeled cholera toxin binding studies showed no change in the number or affinity of cholera toxin receptors in wild type or kin- cells grown under the two conditions. In cell homogenates, wild type cells grown in serum-containing medium had 28-45% higher activity of cyclic nucleotide phosphodiesterase than did cells grown in serum-free medium. Growth with or without serum produced no change in the Km values (14 and 0.25 uM, respectively, for the 2 forms) of phosphodiesterase in these cells. Basal, NaF-, Gpp(NH)p-, and isoproterenol plus GTP-stimulated adenylate cyclase activities were similar in crude plasma membrane preparations from serum-free and serum-grown wild type cells. Horse serum contained a nondialyzable (greater than 8,000 daltons), nonlipid factor which depressed the isoproterenol-stimulated cAMP accumulation of wild type S49 cells and which required 3-4 hr to produce this effect. This factor appears to act via the nucleotide-coupling unit of the adenylate cyclase system and protein kinase, and ultimately results in an increase in cyclic nucleotide phophodiesterase activity. We suggest that studies of the cAMP pathway in S49 cells should ideally be carried out under the less perturbing con ditions offered by serum-free medium. (Author abstract) (35 Refs)

22. BETA-ADRENERGIC RECEPTOR IN MAMMALIAN PLACENTA: COMPARISON OF RAT, GUINEA PIG, RABBIT, HUMAN AND TROPHOBLASTIC TUMORS (MEETING ABSTRACT).

Moore J, Whitsett JA

Department of Pediatrics, University of Cincinnati College of Medicine

Pediatr Res; 15(4,Part2):485 1981

Beta-Adrenergic receptor (beta AR) has been previously demonstrated in human placental membranes. Its demonstration in placentas of multiple species and trophoblastic tumors might support its regulatory function on the trophoblastic cell itself. Beta AR in rat, guinea pig, rabbit and human placenta and trophoblastic tumors were therefore examined. Term placentas were obtained, dissected to remove decidua and processed in identical fashion. The beta-adrenergic antagonist dihydroalprenolol, (-)[3H]DHA bound to a single, saturable class of sites in all placental membranes. Placental beta AR was coupled with catecholamine sensitive adenylate cyclase in all species. Beta AR was absent in two human choriocarcinoma cell lines (JEG-3, BeWo) but was present in a hydatidiform mole obtained freshly from surgery. We conclude that 1) variable but

consistently high density beta AR is present coupled to adenylate cyclase in mammalian placenta, 2) the affinity (KD) of the beta AR is similar in all species; and 3) the presence of high denisty beta AR on a hydatidiform mole containing almost totally trophoblast tissue suggests a beta AR is located on trophoblastic cell membranes. (no Refs)

23. AN IMMUNOLOGIC LOOK AT EARLY MOUSE DEVELOPMENT.

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Dept. Biology, Johns Hopkins Univ., Charles and 34th St., Baltimore, MD, 21218

Cell Immunol; 62(2):247-250 1981

Studies of the surface antigens on normal mouse embryos and teratocarcinomas are summarized. The antigens might provide receptors for cell positioning in normal development, and they could also be receptors for growth-stimulating immune responses by pregnant animals. Many of these cell surface antigens appear to be glycolipids. (18 Refs)

TRANSPORT STUDIES

24. REGULATION OF PASSIVE POTASSIUM TRANSPORT OF NORMAL AND TRANSFORMED 3T3 MOUSE CELL CULTURES BY EXTERNAL CALCIUM CONCENTRATION AND TEMPERATURE.

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J Membr Biol; 61(3):155-172 1981

Regulation of passive potassium ion transport by the external calcium concentration and temperature was studied on cell cultures of 3T3 mouse cells and their DNA-virus transformed derivatives. Upon lowering of external calcium concentration, passive potassium efflux generally exhibits a sharp increase at about 0.1 mM. The fraction of calcium-regulated potassium efflux is largely independent of temperature in the cases of the transformed cells, but shows a sharp increase for 3T3 cells upon increasing temperature above 32 C. In the same range of temperature, the 3T3 cells exhibit the phenomenon of high-temperature inactivation of the residual potassium efflux at 1 mM external calcium. At comparable cellular growth densities, the transformed cell lines do not show high-temperature inactivation of 'residual' K efflux. These results are consistent with the notion of a decisive role of the internal K+ concentration in the cell-density dependent regulation of cell proliferation. In particular, the growth-inhibiting effect of lowering the external Ca++ concentrations is considered as largely due to a rise of passive K+ efflux and a subsequent decrease of internal K+ concentration. The experimental data on the Ca++ dependence of passive K+ flux are quantitatively described by a theoretical model based on the constant field relations including negative surface charges on the external face of the membrane, which cooperatively bind Ca++ ions and may concomitantly undergo a lateral redistribution. The present evidence is consistent with acidic phospholipids as representing these negative surface charges. (Author abstract) (111 Refs)

25. SURPRISING DIFFERENCES IN SUBSTRATE SELECTIVITY AND OTHER PROPERTIES OF SYSTEMS A AND ASC BETWEEN RAT HEPATOCYTES AND THE HEPATOMA CELL LINE HTC.

Handlogten ME, Garcia-Canero R, Lancaster KT, Christensen HN (c/o Christensen) Dept. Biological Chemistry, Univ. Michigan, Ann Arbor, MI, 48109

J Biol Chem; 256(15):7905-7909 1981

We find that the two wide-range Na+-dependent transport systems A and ASC for various neutral amino acids can be discriminated more sharply in the hepatoma cell line HTC than in any cell yet studied by us in which the two systems co-exist. The gain comes partly from a higher reproducibility and a higher relative ASC rate for HTC than in ordinary rat hepatocytes, also a repressed condition of System A unless first deprived of amino acids, but mainly from our finding that in the hepatoma cell threonine serves as a nearly specific substrate and inhibitor of System ASC, thus decisively supplementing older discriminatory techniques. In ordinary hepatocytes cysteine is quite specific to ASC as a substrate but not as an inhibitor, whereas threonine is specific in neither role. In the hepatoma cell cysteine in turn is specific in neither role. In addition to these and other differences between the two cells in analog specificity, which are partly assignable to System ASC and partly to System A, System ASC of the hepatoma cell shows an inhibition on lowering the pH from 6.5 to 5 not seen in the ordinary hepatocyte. Furthermore, threonine uptake by the hepatoma cell undergoes no stimulation when Li+ is substituted for choline in a Na+-free medium, whereas ASC uptake by the ordinary rat hepatocyte is stimulated much as is System A uptake. As in other oc currences, and in contrast to System A, ASC transport in the hepatoma cell is stimulated neither by amino acid deprivation nor by insulin, glucagon, or dexamethasone. Trans-stimulation, both inward and outward, via System ASC is vigorous in the hepatoma cell. Despite the surprising differences observed, common features of each system in various occurrences continue to justify the use of the abbreviations ASC and A as long as they are understood as generic designations. (Author abstract) (15 Refs)

26. EVIDENCE FOR ACTIVATION OF AN ACTIVE ELECTROGENIC PROTON PUMP IN EHRLICH ASCITES TUMOR CELLS DURING GLYCOLYSIS.

Heinz A, Sachs G, Schafer JA

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J Membr Biol; 61(3):143-153 1981

The addition of glucose to a suspension of Ehrlich ascites tumor cells results in rapid acidification of the extracellular medium due to lactic acid production. The nature of the H+ efflux mechanism has been studied by measuring the time course of the acidification, the rate of proton efflux, the direction and relative magnitude of the H+ concentration gradient, and the voltage across the membrane. Using the pH-sensitive dye acridine orange, we have established that after addition of 10 mM glucose an outward-directed H+ concentration gradient develops. As the rate of glycolysis slows, the continued extrusion of H+ reverses the direction of the H+ concentration gradient. Changes in absorbance of the voltage-sensitive dye diethyloxadicarbocyanine iodide (DOCC) and changes in the distribution of the lipid permeant cation tetraphenyl phosphonium, showed a dramatic and persistent hyperpolarization of the membrane voltage after glucose addition. The hyperpolarization was prevented by the protonophore tetrachlorosalicylanalide (TCS) and by valinomycin, but not by

the neutral-exchange ionophore nigericin. Inhibitors of lactate efflux were found to reduce the rate of acidification after glucose addition but they had no effect on the magnitude of the resulting hyperpolarization. On the basis of these and other data we suggest that an active electrogenic pump mechanism for H+ efflux may be activated by glucose and that this mechanism operates independently of the lactate carrier system. (Author abstract) (24 Refs)

27. STEROID SPECIFICITY OF THE GLUCOCORTICOID INHIBITION OF AMINO ACID TRANSPORT IN RAT HEPATOMA CELLS.

Gelehrter TD, McDonald RA
Dept. Human Genetics Box 015, Univ. Michigan Medical Sch., Box 015, Ann Arbor, MI, 48109
Endocrinology; 109(2):476-482 1981

Dexamethasone, a synthetic glucocorticoid, inhibits the initial rate of transport of the nonmetabolizable amino acid, alpha-aminoisobutyric acid, in rat hepatoma tissue culture (HTC) cells. To determine whether this inhibition is mediated by the same proximal steps as is the steroidal induction of tyrosine aminotransferase, we have examined the hormonal specificity of these two responses for various steroids previously characterized with respect to transaminase induction as agonists, partial agonists, antagonists, or inactive steroids. We conclude that the steroidal inhibition of amino acid transport, at steroid concentrations of 10-5M or less is mediated by the same glucocorticoid receptor mechanisms as the induction of tyrosine aminotransferase. First, the concentrations at which the full agonists, dexamethasone and cortisol, produce their half-maximal effects on both phenomena are the same. Second, tetrahydrocortisol, which does not interact with the glucocorticoid receptor, neither inhibits transport nor induces transaminase. Third, the competitive interactions between partial agonists or antagonists and the full agonist dexamethasone with respect to both transport inhibition and enzyme induction are virtually identical. At concentrations greater than 10-5M, however, both partial agonist and antagonist steroids are capable of fully inhibiting amino acid transport. The effects of these steroids on transport, like those of dexamethasone, are reversible and are blocked by cycloheximide and actinomycin D. Furthermore, these steroids, like dexamethasone, slow the rate of efflux of alpha-aminoisobutyric acid from preloaded cells. Thus, the effects of high concentrations of partial agonist and antagonist steroids on amino acid transport do not appear to reflect a generalized toxic effect on membrane function. (Author abstract) (18 Refs)

28. DIFFERENCE IN PUTRESCINE TRANSPORT IN UNDIFFERENTIATED VERSUS DIFFERENTIATED MOUSE NB-15 NEUROBLASTOMA CELLS.

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Dept. Chemistry, Wright-Rieman Lab., Rutgers Univ., Piscataway, NJ, 08854
Biochem Biophys Res Commun; 101(1):243-249 1981

The transport rates of the polyamine putrescine into nondifferentiating (NB) and cyclic AMP-induced differentiating (ND) neuroblastoma cells in tissue culture were measured and compared over a 5 day period. The differentiation of ND cells was initiated by adding 1 millM dibutyryl cyclic AMP (Bt2cAMP) and 0.5 milliM 3-isobutyl-1-methyl (IBMX) xanthine to the normal growth medium. The rate of transport (14C-putrescine uptake over a 20 min incubation period) in ND cells showed a 27% decrease when compared to that of the NB control 6 hr after Bt2cAMP and IBMX addition, and remained significantly lower than that of the control over the entire 5 day period. The addition of Bt2cAMP to the experimental medium during the transport measurement had no effect on putrescine transport, indicating that transport inhibition was not due to a direct cAMP effect. The decrease of putrescine transport was specific: concomitant measurements of aminoisobutyric acid (AIB) transport revealed an inhibition of only approx 3% in ND compared to NB cells. T he Vmax and Km of NB cell putrescine transport, taken at the early stationary phase of growth (time = approx 80 hr), were 5.3 nanomole/mg protein/hr and 2.75 uM respectively. In ND cells the Vmax was identical to that for NB cells while the Km was 10x greater (28.5 uM). The specific inhibition of putrescine transport in ND cells may represent one of the earlier changes of membrane function associated with cell differentiation. (24 Refs)

MEMBRANE BIOGENESIS AND METABOLISM

29. DIFFERENTIATION OF CELL SURFACE RECEPTORS ON NORMAL HUMAN BONE MARROW MYELOID PRECURSORS.

Barrett SG, Hansen KS, Bainton DF
Cancer Res. Inst., Univ. California, San Franciso, CA, 94143
Br J Haematol; 48(3):491-500 1981

Receptors for IgG and complement are present on the plasma membrane of functionally mature blood neutrophils. Neutrophilic precursors lack these membrane receptors, which may account for their diminished phagocytic capability. In this study, myeloid precursors from human bone marrow were examined for the presence and activity of membrane receptors for IgG and activated complement. Morphologic identification by light microscopy of neutrophilic cells was made by rosetting techniques and chloroacetate esterase cytochemical staining and at the ultrastructural level by peroxidase reactivity. This study showed that the Fc receptor was the only membrane marker on the myeloblast and that the number of cells with this receptor increased with maturation. Functional investigation of the Fc receptor indicated that its presence on early myeloid cells was insufficient to induce IgG-mediated ingestion. Phagocytosis via the Fc receptor was not acquired until the metamyelocyte stage. Complement receptors, C3d and C3b, appeared later than the Fc receptor. The C3d receptor, detected first on the promyelocyte, remained relatively constant throughout maturation, whereas the C3b receptor acquired at the myelocyte stage increased with maturation. These data provide evidence that distinct membrane changes occur during normal myeloid cell maturation and that the presence of these membrane receptors is not sufficient for Fc-mediated ingestion to occur. (Author abstract) (28 Refs)

30. IMMUNOLOGIC DISSECTION OF THE ANTIGENIC SPECIFICITY INDUCED BY ROUS SARCOMA VIRUS BY MEANS OF MONOCLONAL ANTIBODIES (MEETING ABSTRACT).

Ferracini R, Comoglio PM
Istituto di Anatomia Umana, Universita di Torino, Turin, Italy
Tumori; 67(2,SupplA):167 1981

Rous sarcoma virus (RSV)-transformed mammalian fibroblasts express a membrane antigen (VCSA) controlled by trans-

forming gene src, the viral genome segment directly responsible for the induction and maintenance of neoplastic transformation. By cloning somatic hybrids of murine myeloma lines with splenic lymphocytes from mice hyperimmunized with RSV-transformed cells, it has been possible to produce a certain number of monoclonal antibodies directed against this antigen. Binding tests between selected monoclonal antibodies and a series of cells from different animal species transformed by RSV have made it possible to show that the VCSA antigen is the result of the combined expression on the cell surface of various antigenic determinants controlled either by host cell genes or viral src genes. It is concluded that the expression of VCSA antigen is not due to the insertion of an individual viral protein on the cell surface, but is the result of a change induced by the product of the src gene on one or more components of the plasma membrane coded by cellular genes. (1 Ref) (In ITA)

31. RAPID TURNOVER OF ARACHIDONYL-PHOSPHATIDYLINOSITOL IN L CELLS STIMULATED BY ANTIBODY.

Shearer WT, Richards JE
Dept. Pediatrics, Baylor Col. Medicine, Houston, TX, 77030
Biochem Biophys Res Commun; 101(3):800-806 1981

The incorporation of 14C-arachidonic acid into certain phospholipid fractions of antibody-stimulated mouse tumorigenic methylcholanthrene transformed (L) cell membranes was studied. Organic solvent cell extractions were separated by two-dimensional chromatography and counted for radioactivity by scintillation spectrometry. A clear dose-response relationship was found between IgG binding on and 32P incorporation into intact L cells; incorporation of 32P into an isolated phosphatidylinositol (PI) fraction of L cell membrane was increased by 56%. The degree of chromatographic separation of individual phospholipids indicated the relative rabbit L cell antibody-enhanced incorporation of 14C-arachidonic acid into the PI fraction (53% increase), a change which occurred without corresponding increase in 3H-glycerol incorporation. Two other isolated lipid fractions, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), showed one half or less the PI 14C-arachidonic acid turnover enhancement. PI exhibited a 4.6 and 7.2 x more rapid turnover than PC and PE, respectively. The rapid incorporation of arachidonic acid into the PI membrane fraction may be an important initial event in the antibody-mediated immunological enhancement of tumor cell growth. (15 Refs)

CELL ADHESION

32. EFFECTS OF RETINOIDS ON NEOPLASTIC TRANSFORMATION, CELL ADHESION, AND MEMBRANE TOPOGRAPHY OF CULTURED 10T1/2 CELLS.

Bertram JS, Mordan LJ, Blair SJ, Hui S
Dept. Experimental Therapeutics, Roswell Park Memorial Inst., Buffalo, NY, 14263
Ann NY Acad Sci; 359:218-236 1981

The effects of retinoids on cell biology was studied in readily transformable C3H/10T1/2 CL8 cell line of mouse embryo fibroblasts. Included are studies on membranes and membrane interactions (cell/substrate adhesion, cell spreading, surface topography). Retinyl acetate caused a dose-dependent reversible inhibition of neoplastic transformation induced by 3-methylcholanthrene. Similar effect was recorded for retinol and retinaldehyde, while all-trans- and 13-cis-retinoic acids failed to inhibit transformation of 10T1/2 cells. Retinoids had no effect on the growth of established cells after the phenotypic expression of transformation. Analysis of the effect of retinoids on cell/substrate adhesion showed that retinyl acetate induced a dose-dependent decrease in the rate of release of trypsin-treated cells. The synthetic retinoids showed an extremely variable response in the adhesion assay. Retinyl acetate at 0.5 ug/ml increased the cell spreading as evidenced by a threefold increase in upper cell surface area and a pronounced increase in the percent of flattened cells. (29 Refs)

33. ANODIC MIGRATION OF ACTIN AND INCREASED LEVELS OF A CATHODIC FIBRONECTIN AMONG THE CYTOSKELETAL CHANGES THAT CORRELATE WITH INCREASED CELL ADHERENCE IN LIVER EPITHELIOID TUMOR CELLS.

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Cell Biol Int Rep; 5(8):791-796 1981

Molecular alterations in cytoskeletal organization were examined in R72/3 liver epithelioid tumor cells which become more adherent to their growth substratum upon exposure to the halogenated pyrimidine iododeoxyuridine (IUDR). Cells were grown for 72 hr in the presence of 2.5 ug/ml IUDR, then extracted successively with 1% and 0.5% Triton-X-100 detergent. Once IUDR had been shown to promote cell adherence (in EDTA-treated cultures, 37% versus 73% of control cells were detached; without EDTA 2.5% versus 4% of controls were detached), simultaneous two-dimensional analysis was performed on samples solubilized in 8 M urea. Isoelectric focusing with Ampholines in the pH range 3-10 followed by polyacrylamide gel co-electrophoresis in the same slab showed clear differences with respect to non-IUDR-exposed controls. Seen were an increase in the expression of a 220,000 (220K) dalton component towards the cathode, only an anodic 64K dalton species, a decrease in the more anodic of three species appearing in the 50K dalton region, and most actin-like 44K dalton components migrating closer to the anode. Assay for fibronectin in the two-dimensional gel revealed that the 230K component detected in IUDR-treated cells is antigenically related. The data suggest an involvement of increased fibronectin levels in the adherence observed in IUDR-treated cells. The presence of both fibronectin- and actin-like molecules among the cytoskeletal components is confirmed. The results indicating that levels of a cathodic fibronectin increase under conditions in which actin becomes more anodic suggest that the two macromolecules may interact in an electrostatic manner across the plasma membrane in vivo. (16 Refs)

34. STRUCTURAL ANALYSIS OF FIBRONECTINS FROM NORMAL AND TRANSFORMED CELLS (MEETING ABSTRACT).

Ali IU, Rasheed S, Hunter T
Meloy Laboratories, Rockville, MD
RNA Tumor Viruses, May 20-24, 1981, Cold Spring Harbor, New York. Cold Spring Harbor Laboratory 294 pp., 1981.

Partial proteolysis as well as tryptic peptide fingerprinting suggest that secreted fibronectins from normal hamster and rat cells and their virus-transformed derivatives have very similar primary structures. There are also regions of extensive homol-

ogy between fibronectins of different species. Analysis of phosphoamino acids of fibronectin showed that phosphorylation occurs at very specific and limited number of sites, all of which are serine residues. Although phosphorylation of fibronectin occurs at the same sites in both normal and transformed cells, fibronectin from transformed cells is phosphorylated to a much greater extent than that from normal cells. This suggests phosphorylation of both chains of fibronectin in transformed cells as opposed to only one in normal cells and/or phosphorylation of a higher fraction of the fibronectin molecules in transformed cells. (no Refs)

35. MEMBRANE GLYCOPROTEINS AND FIBRONECTIN-MEDIATED CELL ADHESION (MEETING ABSTRACT).

Galetto G, Comoglio P, Tarone G
Universita di Trieste, Trieste, Italy
Tumori; 67(2,SupplA):179 1981

The reduced capacity of neoplastic cells for adhesion to the extracellular matrix is one of the important factors critical in determining the metastatic properties of these cells. The adhesion of fibroblasts to their substrate is mediated by fibronectin, a high mol wt glycoprotein which acts as a bridge between the collagen fibers of the matrix and an as yet unknown receptor(s) on the cell membrane. Identification of this receptor(s) is extremely important to understanding the changes in adhesiveness of neoplastic cells. In this paper we investigated the effects of proteolytic digestion of membrane glycoproteins on BHK fibroblast adhesion to culture dishes coated with fibronectin. Pronase digestion (1 mg/ml for 20 min at 37C) destroys the capacity of BHK cells to adhere and flatten out on a fibronectin substrate. On the other hand, prolonged digestion with trypsin (1 mg/ml 20 min at 37C) is totally ineffective, indicating that the receptors(s) for adhesion to fibronectin is protein in nature but insensitive to tryptic digestion. In order to identify these trypsin-resistant molecules, we obtained a direct antiserum against the plasma membrane of BHK cells. This antiserum inhibits the adhesion of trypsin-treated cells to a fibronectin substrate. Furthermore, immunoprecipitation experiments indicate that these antibodies recognize 2 glycoproteins with mol wt of 130 Kd and 80 Kd present on the surface of the trypsinized cells, but not the pronase-treated cells. (no Refs) (In ITA)

OTHER RELATED STUDIES

36. SPECIFIC AND NONSPECIFIC ALTERATIONS IN MEMBRANE MICROVISCOSITY INDUCED BY RETIN OIDS IN EMBRYONAL CARCINOMA AND FIBROBLAST CELLS.

Jetten AM, Meeks RG, De Luca LM
Lab. Experimental Pathology, NCI, NIH, Bethesda, MD, 20205
Ann NY Acad Sci; 359:398-400 1981

The effects of retinoids on the membrane microviscosity of some eukaryotic cells was measured by fluorescence polarization analysis with the aid of the fluorescent probe 1,6-diphenyl 1,3,5-hexatriene. In cultured 3T6 fibroblasts, retinoic acid (RA) at concentrations of up to 10(-7) M had no influence on microviscosity while higher concentrations caused a marked decrease in viscosity. Retinoids also decreased the microviscosity of mouse fibroblast 3T12, 3T3 and 3T3 SV cells. Embryonal carcinoma cells showed an increase in microviscosity during differentiation induced by RA. After 24 hr of treatment with RA (10(-7) M), membrane viscosity increased steadily, concomitantly with the appearance of cells with a fibroblastic morphology. This increase is only caused by analogs that are able to induce differentiation. At high concentrations retinoids caused a decrease in microviscosity of the membranes of mouse fibroblast cells; this change occurred with both biologically active and inactive analogs, indicating the nonspecific character of retinoids. In the embryonal carcinoma cells only biologically active retinoids had an effect on microviscosity. (8 Refs)

37. CHANGES IN HELA CELL PROLIFERATION RATE AND CELL SURFACE COMPONENTS.

Lotan R, Kramer RH, Nicolson GL
Dept. Developmental and Cell Biology, Univ. California, Irvine, CA, 92717
Ann NY Acad Sci; 359:407-409 1981

Biochemical changes induced by retinoic acid (RA) and their possible involvement in growth control of epithelial cells were studied in cultured HeLa cells. The results showed that RA modifies HeLa-S3 cell proliferation, morphology and cellular glycoprotein structure. (11 Refs)

38. INHIBITION PROPERTIES OF SEPHAROSE-BOUND TRYPSIN AND A PROTEASE ON THE SURFACE OF EHRLICH ASCITES TUMOUR CELLS.

Steven FS, Griffin MM, Itzhaki S, Al-Habib A
Dept. Medical Biochemistry, Univ. Manchester, Stopford Building, Manchester M13 9PT, England
Biochim Biophys Acta; 660(2):333-340 1981

Ehrlich ascites cells have been shown to possess a protease with beta-naphthylamidase activity located on the surface of these cells. This enzyme is protected from the inhibitory action of protein inhibitors of trypsin (EC 3.4.21.4) in free soln, but is inhibited by high concentrations of active site-directed inhibitors of trypsin. We believe the protection against inhibition is provided by the location of this protease on the cell surface. We employed a model system of trypsin coupled to Sepharose to demonstrate the protective action of an inert surface, resulting in a marked reduction in inhibition of trypsin-Sepharose, compared to trypsin in free soln, when exposed to both high and low mol wt inhibitors. This cell surface protease has been shown to play a role in activation of the zymogen of collagenase exported by tumor cells. This role may have important implications for tumor cell invasion of the intercellular matrix. (Author abstract) (17 Refs)

39. LIVER PLASMA MEMBRANES AND PROTEOGLYCAN PREPARED THEREFROM INHIBIT THE GROWTH OF HEPATOMA CELLS IN VITRO.

Kawakami H, Terayama H
Zoological Inst., Faculty Science, Univ. Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan
Biochim Biophys Acta; 646(1):161-168 1981

Plasma membranes from rat liver or kidney inhibited the growth of hepatoma (AH-130) cells in vitro. AH-130 plasma membranes or RBC ghosts inhibited the growth of AH-130 cells less effectively. The inhibitory activity of liver plasma membranes was lost by heat treatment, or mild protease (papain or bromelin, but not trypsin or pronase) treatment, whereas it was retained after sialidase treatment or delipidation by ethanol/ether. Proteoglycan (proteoheparin sulfate) prepared from liver plasma membranes inhibited the growth of

AH-130 cells, but heparin sulfate was less active. The inhibitory activity of liver plasma membranes seemed, however, not to be ascribable solely to proteoheparin sulfate associated with plasma membranes. Preliminary investigations suggested that the mol wt 40,000 component may be a major inhibitory principle in liver plasma membranes. (Author abstract) (18 Refs)

40. ROLE OF AEROBIC CONDITIONS IN THE CONTROL OF CELL PROLIFERATION.

Karlberg I, Lindahl-Kiessling K, Low H, Mattsson A
(c/o Lindahl-Kiessling), Inst. Zoophysiology, PO Box 560, S-751 22 Uppsala, Sweden
Int Arch Allergy Appl Immunol; 65(3):250-256 1981

Our experiments demonstrate that proliferation of human lymphocytes as well as fibroblasts is inhibited by oxygen. It is not a toxic effect and it has not been possible to find an intermediary link in intracellular glycolysis, oxidative metabolism, or macromolecule synthesis. Accessory cells may reduce the inhibitory action of oxygen, thereby exerting an influence on the growth rate. In view of recent hypotheses on the mediation of hormone effects via a membrane dehydrogenase system our results may indicate that also the mitotic activation is dependent on the ***redox state of the membrane*** for the function of a proton gradient across the plasma membrane itself, delivering energy for glucose and amino acid transport. (Author abstract) (21 Refs)

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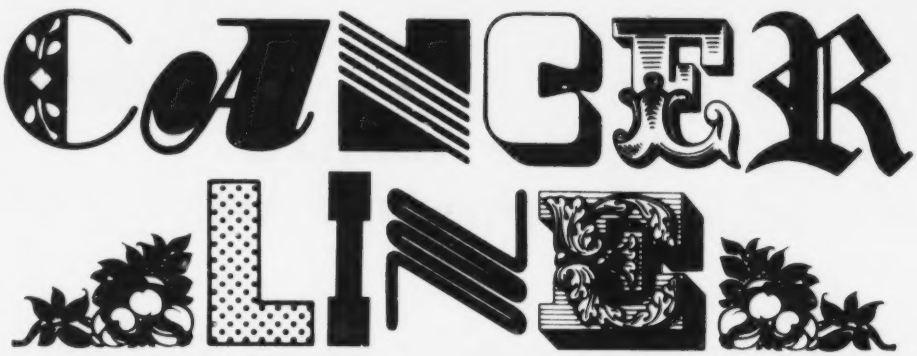

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STRUCTURAL AND FUNCTIONAL ASPECTS OF CELL MEMBRANES

SERIES CB06 No. 82/02

FEBRUARY, 1982

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. METASTATIC POTENTIAL CORRELATES WITH CELL-SURFACE PROTEIN ALTERATIONS IN B16 MELANOMA VARIANTS.

Rieber M, Rieber MS
Centre Microbiology and Cell Biology, Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas 1010A, Venezuela
Nature; 293(5827):74-76 1981

Mice cell lines of differing metastatic potential in an in vivo assay (im injection into 9-12-wk-old C57BL mice of 2.5 x 10(4) cells from subconfluent cultures) were studied: B16-F10Lr cells (less than 5% pulmonary metastasis), B16-F10 cells (25-33% multiple lung metastases), and B16-BL6 (60-75% pulmonary metastasis); F10 cells were derived from F10Lr cells by successive in vivo/in vitro passaging, and BL6 cells were derived

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from F10 cells by selection for increased invasive capacity. Treatment with 1 M urea following enzymatic pre-iodination of cell-surface proteins and subsequent electrophoretic analysis showed low mol wt (approx 18,000 [18 K]) and high mol wt (approx 78 K) components from F10Lr cells, but only the high mol wt components from F10 cells. However, 8 M urea extracts of pre-iodinated F10Lr cells gave lower levels of the fast-migrating (18 K) protein, did not reveal the presence of several fast-migrating components, and showed the presence of slower-migrating components; this result may be due to the release or activation of a protease from the nonmetastatic cells by 1 M urea that may be inhibited by 8 M urea. Electrophoresis following the promotion of membrane vesiculation on surface proteins with p-formaldehyde and dithiothreitol revealed similar patterns in the two metastatic cell lines but a different pattern in the low-metastasis line; the latter showed an 18 K species, the absence of a 45 K species, and the presence of a significant species that migrated slightly faster than the 78 K species of the metastatic lines. Clones of the three cell lines at limiting dilutions revealed that the demonstrated differences were retained in the clones. Extraction of surface-iodinated cells with Triton-X followed by electrophoresis, revealed that the Triton-insoluble cell-surface components of the metastatic cell lines gave similar patterns with comparable amount of the 78 K species, the low-metastasis cell line had a greater level of radioactivity in the fast-migrating species. This is apparently the first report of cell-surface protein differences between cells of different metastatic potential. (16 Refs)

2. ISOLATION AND PARTIAL CHARACTERIZATION OF A MUCIN-TYPE GLYCOPROTEIN FROM PLASMA MEMBRANES OF HUMAN MELANOMA CELLS.

Umemoto J, Bhavanandan VP, Davidson EA
(c/o Bhavanandan) Dept. Biological Chemistry, Milton S. Hershey Medical Center, Pennsylvania State Univ., Hershey, PA, 17033
Biochim Biophys Acta; 646(3):402-410 1981

Plasma membranes were isolated from HM7 melanoma cells grown in the presence of [3H]glucosamine and Na35/2SO4 or [3H]mannose and [14C]glucosamine. The labeled glycoconjugates were solubilized with 0.6 M lithium diiodosalicylate/0.5% Triton X-100. Fractionation of glycoconjugates by repeated chromatography on columns of Sepharose CL-6B and DEAE-Sepharose and by affinity chromatography on WGA-Sepharose yielded three radiochemically homogenous glycoproteins. One of these having an apparent mol wt of 100,000 was found to contain clusters of (AcNeu)1 or 2--[Gal--GalNAc] linked O-glycosidically to the protein. One other glycoprotein contained both O-glycosidically and N-glycosidically-linked oligosaccharides, and the third contained only N-glycosidically-linked carbohydrates. Preliminary results indica te that the 100,000 mol wt mucin-type glycoprotein is present in significantly reduced quantities in cultured human fetal uveal melanocytes. Further, the bulk of the glycoproteins from the melanocytes were of lower molecular size compared to those from the melanoma cells. (Author abstract) (33 Refs)

3. BIOCHEMICAL ANALYSIS OF NORMAL HUMAN TISSUES AND THOSE OF LYMPHOMA PATIENTS BY HIGH-RESOLUTION PROTEIN MAPPING.

Westerbrink K, Warthold U, Hebbeln J, Postel E, Havsteen B
Inst. Biochemistry, Sch. Medicine, Univ. Kiel, Olshausenstrasse 40-60, D-2300 Kiel, W. Germany
J Cancer Res Clin Oncol; 101(1):43-51 1981

Membrane proteins from various normal human tissues, (tonsils, prostate, etc) and from tissues of lymphoma patients (spleen and lymph nodes) were mapped by high-resolution, two-dimensional electrophoresis. The protein maps showed high tissue specificity for normal tissues, and they were sensitive to pathologic deviations from normal. Changes found in the protein maps of patients with hairy cell leukemia, chronic lymphatic leukemia, immunocytoma, plasmacytoma, and centroblastic centrocytic lymphoma are described. The pattern of proteins from different lymphomas differed more from each other than those of normal tissues, a factor that might be useful in the development of a 'tumor-specific protein code.' The code could be represented by the spatial distribution and relative intensities of proteins stained with coommassie brilliant blue or, in the case of minor proteins, a sensitive silver stain. A protein code would provide the pathologist with an additional set of parameters for tumor identification, characterization, and classification. (11 Refs)

4. DISTRIBUTION AND MOLECULAR CHARACTERIZATION OF A CELL-SURFACE AND A CYTOPLASMIC ANTIGEN DETECTABLE IN HUMAN MELANOMA CELLS WITH MONOCLONAL ANTIBODIES.

Wilson BS, Imai K, Natali PG, Ferrone S
Dept. Molecular Immunology, Scripps Clinic Res. Foundation, 10666 North Torrey Pines Road, La Jolla, CA, 92037
Int J Cancer; 28(3):293-300 1981

The monoclonal antibodies 225.28S and 465.12 to human melanoma-associated antigens have been tested with a large variety of surgically removed skin lesions and malignant tumors as well as with a panel of cultured cell lines in serological and immunochemical assays. The antibody 225.28S reacts with a plasma membrane antigen while the antibody 465.12 detects a cytoplasmic antigen. Both antibodies fail to react with melanocytes from normal skin as well as benign skin lesions but react with nevi, melanoma cells and some skin carcinomas. Analysis with surgically removed tumors and cultured human cell lines indicated that the plasma membrane antigen is restricted to skin lesions whereas the cytoplasmic antigen is synthesized by tumor cells of various histological origins. The plasma membrane antigen is composed of two glyco-polypeptides of 280,000 and greater than 440,000 daltons, while the cytoplasmic antigen consists of four glycopolypeptides of 94,000, 75,000, 70,000 and 25,000 daltons. None of these components are bridged by disulfide bonds. The cytoplasmic antigen was readily detected in the spent culture medium of melanoma cell lines in the form of a major 94,000 dalton and a minor 72,000 dalton structure, while the plasma membrane antigen was detectable only after vastly increasing the sensitivity of the assay system. (Author abstract) (23 Refs)

5. HUMAN TROPHOBLAST CELL-SURFACE ANTIGENS DEFINED BY MONOCLONAL ANTIBODIES.

Lipinski M, Parks DR, Rouse RV, Herzenberg LA
Laboratoire d'Immunologie Clinique, Institut Gustave-Roussy, 94800 Villejuif, France
Proc Natl Acad Sci USA; 78(8):5147-5150 1981

A series of monoclonal antibodies has been raised against the human choriocarcinoma cell-line, BeWo. Four antigens, Trop-1,-2,-3, and-4, are defined on normal and malignant trophoblast cells. Trop-1 and Trop-2 appear to be specifically expressed on syncytio- and cytotrophoblasts, whereas Trop-3 and Trop-4 are also detected on various tumor cell lines, normal lymphocytes, and monocytes. Anti-Trop-1 and and anti-Trop-2 antibodies might prove useful for detection and isolation of fetal trophob-

last cells circulating in pregnant women's blood and for diagnosis and therapy in patients having choriocarcinomas and other germ-cell neoplasms. (Author abstract) (16 Refs)

6. CELL SURFACE ANTIGENS OF HUMAN RENAL CANCER DEFINED BY MOUSE MONOCLONAL ANTIBODIES: IDENTIFICATION OF TISSUE-SPECIFIC KIDNEY GLYCOPROTEINS.

Ueda R, Ogata S-I, Morrissey DM, Finstad CL, Szkudlarek J, Whitmore WF, Oettgen HF, Lloyd KO, Old LJ
Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY, 10021
Proc Natl Acad Sci USA; 78(8):5122-5126 1981

Seventeen monoclonal antibodies derived from fusions with spleen cells of mice immunized with established culture lines of renal cancers identified 9 cell-surface antigenic systems. Six of the systems (gp160, S25, gp120r, gp120nr, gp115, and V1) represent antigens not previously described, the other three systems are related to HLA-A, -B, and -C heavy chain and A and B blood group antigens. The most restricted of the newly described antigens are gp160, S25, and gp120r. These determinants are found only on cells of renal origin, both normal and malignant, and represent differentiation antigens of human kidney. In addition to the difference in the mol wt of two of these antigens, gp160, S25, and gp120r can be distinguished on the basis of differential expression on a panel of cultured renal cancers and normal kidney epithelium and fetal kidney cells. Glycoproteins bearing gp120r share a determinant with renal gp120nr (as indicated by sequential precipitations with monoclonal antibodies that detect gp120r and gp120nr), but g p120nr is found on a broader range of cell types, including fibroblasts and cell lines derived from lung, bladder, and colon cancers. The two other new systems, gp115 and V1, have characteristics of broadly occurring differentiation antigens but can be distinguished from each other and from gp120nr by differences in mol wt, heat stability (V1 is a heat-stable determinant), and differential expression on cell types of diverse origin. (Author abstract) (9 Refs)

7. GLYCOSPHINGOLIPIDS OF K562 CELLS: A CHEMICAL AND IMMUNOLOGICAL ANALYSIS.

Suzuki A, Karol RA, Kundu SK, Marcus DM
(c/o Marcus) Dept. Medicine, Microbiology, Immunology, Baylor Coll. Medicine, Houston, Texas, 77030
Int J Cancer; 28(3):271-276 1981

The K562 cell line, which was established from a patient with chronic myeloid leukemia in blast crisis, was thought to be myeloid, but recent data indicate that it is an undifferentiated erythroid cell line. We have found that the glycosphingolipid content of these cells differs considerably from that of mature RBC. Globotetraosylceramide, the most abundant glycolipid of mature red cells, was not detected in K562 cells, and neither was globotriaosylceramide. The predominant neutral glycolipids of K562 cells are monohexaosylceramides, which are a mixture of glucosyl- and galactosylceramides, and lactotriaosyl- and lactoneotetraosylceramides were also detected. Secondly, gangliosides which contain N-acetylgalactosamine were much more abundant than those containing N-acetylglucosamine in K562 cells, in contrast to RBC. The most abundant ganglioside of K562 cells, GM2, is present in trace quantities in RBC. A third major difference between these two cells lies in their relative proportions of neutral glycolipids and gangliosides. The molar ratio of neutral glycolipids/gangliosides is approx 15:1 in RBC and 1:1 in K562 cells. These striking differences between K562 cells and mature RBC indicate that glycolipids may be useful cell surface markers of normal RBC differentiation, and of erythroleukemias. (Author abstract) (29 Refs)

8. COMPARISON OF LIPID COMPOSITION AND 1,6-DIPHENYL-1,3,5-HEXATRIENE FLUORESCENCE POLARIZATION MEASUREMENTS OF HAIRY CELLS WITH MONOCYTES, AND LYMPHOCYTES FROM NORMAL SUBJECTS AND PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.

Liebes LF, Pelle E, Zucker-Franklin D, Silber R
(c/o Silber) Dept. Medicine, New York Univ. Medical Center, 560 First Ave., New York, NY, 10016
Cancer Res; 41(10):4050-4056 1981

In this report, we compare the lipid composition and fluorescence polarization properties of hairy cells with those of monocytes and lymphocytes from normal subjects and of lymphocytes from patients with chronic lymphocytic leukemia. For hairy cells, the cholesterol content was 4.66 +-1.49 (S.D.) umol/10(9) cells, and the cholesterol/phospholipid ratio was 0.60 +-0.09. These were significantly hig her than the values of normal lymphocytes cholesterol content, (cholesterol content, 2.75 +-0.65 umol; cholesterol/phospholipid ratio, 0.50 +-0.07) or of chronic lymphocytic leukemia lymphocytes (cholesterol content, 1.76 +-0.43 umol; cholesterol/phospholipid ratio, 0.44 +-0.07). Normal monocyte values (cholesterol content, 5.81 +-2.08 umol; cholesterol/phospholipid ratio, 0.59 +-0.06) were similar to those of hairy cells. Using the probe 1,6-diphenyl-1,3,5-hexatriene, the fluorescence polarization value at 25 C for hairy cells was 0.302, compared to the value of 0.259 obtained with chronic lymphocytic leukemia lymphocytes. Intermediate values (0.294) were obtained with normal lymphocytes and monocytes. Fluorescence polarization values were higher in hairy cell membranes than in chronic lymphocytic leukemia lymphocyte membranes, indicating a low fluidity in the former cell, compatible with their higher cholesterol content and cholesterol/phospholipid ratio. These results show that two neoplastic cells, hairy cells and chronic lymphocytic leukemia lymphocytes, differ markedly in membrane fluidity and that a high membrane fluidity does not necessarily occur in neoplasia. (Author abstract) (52 Refs)

9. LIPOPHILIC PROTEINS OF FRIEND ERYTHROLEUKEMIA CELLS.

Semmel M
Groupe 8 du C.N.R.S., Institut Gustave-Roussy, 94800 Villejuif, France
Mol Biol Rep; 7(4):241-245 1981

The lipophilic membrane proteins of Friend mouse erythroleukemia cells (MELC), clone 745A, were processed by radioactive labeling, acidic chloroform-methanol extraction, and ether precipitation, and analyzed by sodium dodecyl sulfate-13% polyacrylamide slab gel electrophoresis, fluorographic spectrophotometry, and thin layer chromatography. The acidic extract contained at least four polypeptides of apparent mol wt 5, 9.5, 14, and 17 kilodalton (kD). Delipidation with ether resulted in the formation of approx 12 polymers with apparent mol wt between 25 and 85 kD. Binding occurred between amino acids, sugars, and phospholipids, particularly phosphatidylinositol and phosphatidylethanolamine, and the polypeptides. The evidence suggests that most of the extracted lipophilic proteins are of cellular origin; part of the polypeptides of mol wt 14 and 17 kD could be viral components. The

formation of polymers upon delipidation distinguishes the polypeptides from other cellular proteins. (16 Refs)

10. ISOLATION AND CHARACTERIZATION OF PLASMA MEMBRANES FROM TRANSPLANTABLE HUMAN ASTROCYTOMA, OAT CELL CARCINOMA, AND MELANOMAS.

Knowles AF, Leis JF, Kaplan NO
Dept. Chemistry, Q-058, Sch. Medicine, Univ. California, San Diego, La Jolla, CA, 92093
Cancer Res; 41(10):4031-4038 1981

Purified plasma membranes were obtained from five transplantable human tumors, a Grade IV astrocytoma, an oat cell carcinoma, and three melanomas. Plasma membrane fractions were isolated fro m tumor homogenates by differential and discontinuous sucrose gradient centrifugation. Determination of enzyme activities indicated that the plasma membranes were enriched 10-to 20-fold with respect to 5'-nucleotidase, nicotinamide adenine dinucleotide glycohydrolase, Mg + +-activated nucleoside triphosphatase, and sialic acid. Specific activities of nearly all the enzymes varied with the individual tumors, even among tumors of the same type, ie, the melanomas. Electron micrographs of the plasma membrane fractions showed smooth single-membrane vesicles with slight contamination by lysosomes. Therefore, these membranes are suitable for comparative biochemical studies and for the preparation of tumor-specific monoclonal antibodies. Plasma membranes from all five tumors contained very high Mg + +-adenosine triphosphatase (ATPase) activities. The Na + -K + -ATPase was a minor component of the total ATPase of these membranes (less than 30%). The major component was an ATPase exhibiting similar activity toward several nucleoside triphosphates. The activity of such a nucleoside triphosphatase has been correlated with tumorigenicity in cultured liver epithelial cells. The nucleoside triphosphatase of the plasma membranes of astrocytoma and oat cell carcinoma was stimulated from 50 to 100% by concanavalin A, whereas ATPase of the melanoma plasma membranes was not or only slightly stimulated. The different response to concanavalin A could be due to differences in the ATPase molecules of the individual tumors or to the different environment of the ATPase. (Author abstract) (35 Refs)

11. STIMULATION BY TUBULIN OF AN ADENYLATE CYCLASE FROM MURINE PLASMACYTOMA.

Simonin G, Zachowski A, Huitorel P, Pantaloni D, Paraf A
Station de Virologie ed d'Immunologie, l'Institut National Agronomique, F-78850 Thirerval-Grignon, Yvelines, France
Eur J Biochem; 118(3):515-519 1981

Different brain tubulin preparations were shown to stimulate membrane-bound adenylate cyclase from the murine plasmacytoma MOPC 173. Purified tubulin devoid of microtubule-associated proteins and of nucleoside-diphosphate kinase activity was responsible for this stimulation. Activation of the basal adenylate cyclase activity occurred in less than 2 min at 32 C and was amplified by a 4 C preincubation of tubulin with plasma membranes. Tubulin affected the Km and the V of the enzyme and was shown to be associated with the membrane during the activation phenomenon. Tubulin was more active on the basal adenylate cyclase activity than that stimulated by fluoride or guanosine 5'-[beta,gamma-imido]triphosphate. GTP has no effect on the tubulin-stimulated enzyme. (Author abstract) (44 Refs)

12. ALTERATION OF MEMBRANE-ASSOCIATED ENZYMES IN DRUG-RESISTANT SUBLINES OF MOUSE LYMPHOBLASTOMA L5178Y CELLS.

Sugimoto Y, Nishimura T, Suzuki H, Tanaka N
Inst. Applied Microbiology, Univ. Tokyo, Tokyo 113, Japan
J Antibiot (Tokyo); 34(9):1200-1205 1981

Activities of marker enzymes for various cell components were studied with extracts of adriamycin-, aclacinomycin A- and bleomycin-resistant cells and with partially purified plasma membrane fractions of aclacinomycin A-resistant cells, in comparison with those of the parental cells. Alkaline phosphodiesterase and Na + K + ATPase activities were observed to alter the drug-resistant sublines, but other enzymes showed similar activities in the resistant cells to those in the parental cells. Alkaline phosphodiesterase activities in all the resistant sublines were higher than those in the parental cells. Na + K + ATPase activities of anthracycline-resistant sublines were lower and that in bleomycin-resistant cell line was higher than that of the parental cells. The adriamycin-resistant cells exhibited the same level of alkaline phosphodiesterase activity with the aclacinomycin A-resistant cells: Vmax was the same with, and the affinity was twice stronger than the parental cells. The bleomycin-resistant cells showed ca 30% Vmax in comparison with the sensitive cells, and 17 fold higher affinity than the parental cells. The current results, concerning changes of membrane-associated enzymes in drug-resistant sublines of L5178Y cells, support the assumption that the resistance is due to alteration of plasma membrane transport systems. (Author abstract) (14 Refs)

13. BIOCHEMICAL CHARACTERIZATION OF SYRIAN HAMSTER CELL SURFACE ALLOANTIGENS. III. HAMSTER ALLOANTISERA IMMUNOPRECIPITATE CLASS II-LIKE, BUT NOT CLASS I-LIKE, MOLECULES.

Streilein JW, Phillips JT, Stein-Streilein J, Proia DA, Duncan WR
Dept. Cell Biology, Univ. Texas Health Science Center at Dallas, Dallas, TX, 75235
Transplantation; 32(2):106-110 1981

Indirect immunoprecipitation of 12 hamster alloantisera, recently produced by mutual immunizations of domestic inbred strains and recently wild, partially inbred lines, demonstrated that all immunoprecipitates comprise only two species of molecules: one of approx mol wt of 29,000 and the other 39,000 daltons. Surface alloantigens were immunoprecipitated from lymphocyte glycoprotein fractions obtained from every hamster strain tested: MHA, UT1, UT2, MIT, and TX/. In no instance was either component detected alone and no other molecules of different mol wt were found. The expression of these cell surface molecules only on spleen and lymph node cells suggests that hamster T cells display cell surface class II homologues. Putative hamster homologues of class I MHC molecules were not identified by any of the antisera. The hypothesis that hamsters possess a chromosomal region, tentatively designated Hm-1, that comprises genetic loci encoding alloantigens detectable by mixed lymphocyte reactivity and by serology, a region that resembl e the I region of murine H-2, is proposed. (27 Refs)

14. IMMUNOBIOLOGICAL PROPERTIES OF 1-BUTANOL-EXTRACTED CELL SURFACE ANTIGENS.

LeGrue SJ, Allison JP, Macek CM, Pellis NR, Kahan BD
Dept. Biochemistry, Surgery, Univ. Texas Health Science Center, Houston, Texas, 77030
Cancer Res; 41(10):3956-3960 1981

Extractions of viable 3-methylcholanthrene-induced murine sarcoma cells (MCA-F and MCA-2A) prepared using single-phase (2.5%) 1-butanol significantly retarded the outgrowth of the homotypic, but not the heterotypic, tumor of syngeneic C3H/HeJ mice. Butanol extracts specifically evoked a delayed hypersensitivity response in tumor-immune syngeneic mice, but not in alloimmune DBA/2J mice. Crude butanol extracts of MCA-F cells did not contain alloantigenic activity, as shown by their inability to block H-2 or Ia-specific antibodies in a complement-dependent cytotoxicity assay. Absorption of these same allospecific reagents with untreated or with butanol-extracted cells indicated that H-2 antigens remain associated with the cell surface during extraction. Thus, butanol appears to release tumor antigens, but not alloantigens, from the cell surface. (Author abstract) (31 Refs)

15. DEMONSTRATION OF A CORRELATION BETWEEN TUMOR CELL H-2 ANTIGEN CONTENT, IMMUNOGENICITY, AND TUMORIGENICITY USING LECTIN-RESISTANT TUMOR VARIANTS.

Dennis JW, Donaghue TP, Carlow DA, Kerbel RS
(c/o Kerbel) Cancer Res. Div., Dept. Pathology, Queen's Univ., Kingston, Ontario K7L 3N6, Canada
Cancer Res; 41(10):4010-4019 1981

Four wheat germ agglutinin-resistant (WGAR) variants of the highly malignant murine tumor MDAY-D2 were examined for membrane alterations which might correlate with their decrease in tumorigenicity described previously. Injection sc of as many as 5 x 10(6) cells of the WGAR variants MDW1 or MDW3 was rejected by the normal syngeneic DBA/2 host, but they grew rapidly and progressively in athymic nude mice, suggesting that a vigorous T-cell-mediated immune response was responsible for rejection of the variants in the immunocompetent host. In contrast, an inoculum of as few as 10(2) MDW4, MDW5, or MDAY-D2 cells gave rise to progressively growing metastatic tumors in normal DBA/2 mice. Hence, the MDW1 and MDW3 variants appeared more immunogenic than did the MDW4, MDW5, or MDAY-D2 parent tumor. Two lines of evidence suggested that all of the WGAR variants and parental tumors shared a common tumor-associated antigen; (a) rejection of MDW1 tumor cells by DBA/2 mice immunized them against a subsequent challenge of either MDAY-D2 or MDW4 cells; (b) antitumor cytotoxic T-lymphocyte (CTL) activity was detected in a 4-hr 51Cr release assay in spleens taken from tumor-bearing mice and restimulated in vitro with mitomycin C-treated tumor cells. MDAY-D2 and WGAR variants were cross-reactive, both as immunogens at the restimulation stage and as CTL ta rgets. Compared to the tumorigenic lines, MDW1 and MDW3 were more effective stimulators of tumor-specific CTL and were superior targets in the CTL assay. Similarly, stimulation of syngeneic DBA/2 spleen cells with trinitrophenol-modified tumor cells showed MDW1 and MDW3 were more effective stimulators of the trinitrophenyl-specific response and were more readily lysed by trinitrophenyl-directed CTL than were the other WGAR variants or MDAY-D2. Separation of microsomal membrane proteins on sodium dodecyl sulfate:polyacrylamide gel electrophoresis revealed an elevated level of two glycoproteins with apparent mol wt of 43,000 and 47,000, present in the microsomes of MDW1 and MDW3. The glycoproteins were identified as H-2D and H-2K, respectively. Absorption of alloantiserum by whole tumor cells indicated that the WGAR variants and MDAY-D2 had similar amounts of H-2 on their cell surface. In contrast, absorption tests, using tumor cell endoplasmic reticulum and plasma membrane fractions, as well as analysis of the membrane fractions by sodium dodecyl sulfate:polyacrylamide gel electrophoresis, showed an increased level of H-2 in the endoplasmic reticulum of the MDW1 and MDW3 variants. The results demonstrate an inverse correlation between the tumorigenicity and both the immunogenicity and cellular H-2 levels of the variant tumor cell lines. (Author abstract) (37 Refs)

16. LOSS OF PERICELLULAR FIBRONECTIN MATRIX FROM HETEROKARYONS OF NORMAL HUMAN FIBROBLASTS AND EPITHELIAL CELLS.

Laurila P
Dept. Pathology, Univ. Helsinki, SF-00290 Helsinki 29, Finland
Exp Cell Res; 135(1):21-29 1981

The expression of fibronectin in heterokaryons of normal human fibroblasts and normal or malignant epithelial cells was studied by indirect immunofluorescence microscopy. fibroblasts and their homokaryons showed a characteristic pericellular fibronectin matrix, whereas both normal (MDCK) and malignant (HeLa) epithelial cells, and their homokaryons, lacked such a matrix. The fibroblast homokaryons also showed a typical strong, perinuclear cytoplasmic, fibronectin-specific fluorescence. This was much weaker or absent in the MDCK and HeLa cells and their homokaryons. When human fibroblasts were fused with either normal or malignant epithelial cells, no pericellular matrix-like, fibronectin-specific fluorescence could be seen in the heterokaryons. Interestingly, however, a distinct intracellular fluorescence was seen in the heterokaryons, indicating continued production of fibronectin. The results of the present study indicate that both malignant and normal epithelial cells, which do not deposit fibronectin matrix, can cause its loss in heterokaryons with fibroblasts. Thus, discontinued fibronectin matrix formation does not point exclusively to malignancy, but may also reflect the state of differentiation of the parental cells. (Author abstract) (35 Refs)

17. FIBRONECTIN AND RELATED TRANSFORMATION-SENSITIVE CELL SURFACE PROTEINS (ONCOLOGY OVERVIEW).

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ICRDB Program Office, NCI, Westwood Building, Room 10A18, Bethesda, MD, 20205
Fibronectin and Related Transformation-Sensitive Cell Surface Proteins (Oncology Overview) Chen, L B, ed This Title is Available Through National Technical Information Service, Springfield, VA, as NTISUB/PB81-923001:; OB-81/01:95pp. 1981

Oncology Overviews are a service of the International Cancer Research Data Bank (ICRDB) Program of the National Cancer Institute. They are retrospective bibliographies containing 100-500 selected abstracts of published research on topics of high interest to cancer investigators. The scope of this Oncology Overview covers the purification, localization, structure, and function of the 220,000 dalton cell surface transformation-sensitive protein fibronectin (also known as LETS protein, CIg, soluble fibroblast antigen, cell surface protein, and zeta protein). The dramatic reduction of cell surface fibronectin which occurs during transformation may become a useful marker characteristic in correlations of tumorigenicity and the transformed phenotype. The expression of fibronectin on a variety of normal and transformed cells, and the control

of expression, are reviewed. Other areas of fibronectin research which are covered include: the influence of mitogenic proteases on fibronectin in relation to cell growth control; fibronectin interactions with collagen and proteoglycan; ultrastructure of intercellular fibronectin matrix; fibronectin's role in cell-cell adhesion, cell-substratum adhesion, cell surface centripetal flow inhibition, cell motility and restoration of cultured cells to normal phenotype; and differentiation-associated alterations in cell surface fibronectin. Also included are studies of the transformation-sensitive cell surface proteins which are relevant to fibronectin research.

MORPHOLOGICAL STUDIES

18. ULTRASTRUCTURAL, CELL MEMBRANE, AND CYTOGENETIC CHARACTERISTICS OF B-CELL LEUKEMIA, A MURINE MODEL OF CHRONIC LYMPHOCYTIC LEUKEMIA.

Slavin S, Weiss L, Morecki S, Bassat HB, Leizerowitz R, Gamliel H, Korkesh A, Voss R, Polliack A
Dept. Medicine, Immunology Res. Lab., Jerusalem, Israel
Cancer Res; 41(10):4162-4166 1981

A murine model of a spontaneous, transplantable BALB/c B-cell leukemia (BCL1) is described. Extreme leukemia and splenomegaly develop in H-2d-compatible recipients of tumor cells. Tumor cells are medium to large lymphocytes that can be transformed in plasmacytoid cells following in vitro stimulation with lipopolysaccharide. Karyotype analysis of transformed tumor cells reveals 36 chromosomes with several monosomies and 7 marker chromosomes. The ultrastructure of the tumor cells was studied using transmission and scanning electron microscopy. Although the appearance of tumor cells seems normal by morphological criteria, an impaired capping ability was documented using the fluorescein-conjugated concanavalin A-binding test. Impaired capping ability was documented before leukemia was overt as early as 1 to 3 days following inoculation of tumor cells. The B-cell leukemia (BCL1) provides a useful murine model for the study of various aspects of human bone marrow-derived malignant disorders. (Author abstract) (28 Refs)

19. DEMONSTRATION WITH MONOCLONAL ANTIBODIES OF AN UNUSUAL MONONUCLEAR CELL INFILTRATE AND LOSS OF NORMAL EPITHELIAL MEMBRANE ANTIGENS IN HUMAN BREAST CARCINOMAS.

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Nuffield Dept. Surgery, Univ. Oxford, John Radcliffe Hosp., Oxford OX3 9DU, England
Lancet; 2(8244):434-438 1981

Monoclonal antibodies to two differentiation antigens normally expressed on the membranes of breast epithelial cells were used in immunofluorescence studies on frozen sections of malignant breast tumors. It was demonstrated that most tumors did not express these antigens on their cell membranes, and it was possible to classify histologically indistinguishable tumors according to whether they expressed the antigens. The absence of these membrane antigens is likely to be a subtle indicator of cellular differentiation and to have metabolic and other functional implications for the tumor. The demonstration that otherwise indistinguishable tumors can be classified in this way therefore has obvious potential for clinical exploitation. In addition, it was demonstrated that the majority of the mononuclear cells in those tumors with intense mononuclear cell infiltrates expressed an antigen not normally found on mature lymphocytes and macrophages. (Author abstract) (11 Refs)

20. IMMUNOPEROXIDASE STUDY OF ALKALINE PHOSPHATASE IN TESTICULAR TUMOR.

Uchida T, Shimoda T, Miyata H, Skikata T, Iino S, Suzuki H, Oda T, Hirano K, Sugiura M
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Cancer; 48(6):1455-1462 1981

Indirect immunoperoxidase staining was carried out on human testicular tumors using monospecific antibodies against placental (Regan) and intestinal isoenzymes of alkaline phosphatase (ALPase). The very high incidence of seminoma (approx 90%) revealed positive staining of placental ALPase mainly on the cell membrane of tumor cells, whereas none of the seminoma showed presence of intestinal isoenzyme. Placental isoenzyme was not recognized in any embryonal carcinoma and interstitial cell tumor. The epithelial cells of the glandular elements of teratoma occasionally exhibited strong staining for intestinal ALPase and weak staining for placental ALPase. The appearance of Regan isoenzyme in seminomas might be considered possible conversion of hepatic to placental isoenzyme, a consequence of malignant transformation of spermatogenic cells. Regan isoenzyme appears to be a new tumor marker for seminoma and the frequent identification of Regan isoenzyme in seminoma may disclose a unique biologic characteristic of this germinal tumor. (Author abstract) (35 Refs)

21. IMMUNOCYTOCHEMICAL DEMONSTRATION OF INTRACYTOPLASMIC ALKALINE PHOSPHATASE IN HELA TCRC-1 CELLS.

Tokumitsu S, Tokumitsu K, Fishman WH
La Jolla Cancer Res. Foundation, La Jolla, CA, 92038
J Histochem Cytochem; 29(9):1080-1087 1981

The ultrastructural localization of alkaline phosphatase has been examined in cells of a HeLa subline (TCRC-1) that are monophenotypic for Regan isoenzyme expression. Enzyme activity was demonstrated at the cell surface plasma membrane and in certain lysosomes as revealed by the lead citrate method. The regular direct immunoperoxidase procedure utilizing antibodies in IgG or Fab' form showed the same distribution patterns of alkaline phosphatase. However, when the cell surface antigen was blocked in advance with specific unlabeled antibodies and direct immunocytochemistry performed in the presence of saponin, intracellular alkaline phosphatase antigen was observed in the perinuclear space, endoplasmic reticulum, and Golgi apparatus. The results appeared to be concordant with the current concept that membrane glycoproteins are formed in the endoplasmic reticulum, modified in the Golgi apparatus and then transported to the cell surface. Intracellular alkaline phosphatase was observed predominantly in some cell populations especially mitotic cells, suggesting that the enzyme protein was synthesized in and around the mitotic phase. Accordingly, this technique of differential membrane immunocytochemistry appears to provide an opportunity to follow ectopic gene expression as a function of cell cycle and enzyme induction. (Author abstract) (28 Refs)

22. FIBRONECTIN. IMMUNO-MORPHOLOGICAL STUDY OF ITS DISTRIBUTION IN HUMAN PATHOLOGY.

Birembaut P, Gaillard D, Adnet J-J, Dousset H, Kalis B, Poynard J-P, Leutenegger M, Labat-Robert J, Robert L

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Ann Pathol; 1(2):140-146 1981

Fibronectin (FN) is a major glycoprotein component of extra-cellular matrix. This glycoprotein seems to be involved in cell to cell interaction and in cell to extracellular matrix interaction. Its distribution has been studied in various pathological conditions by an indirect immunofluorescent technique using a rabbit anti-FN serum. In various carcinomas, there is a loss of FN normally present within basement membranes, related to tumoral infiltration. Of particular interest is the irregular labeling of basement membranes in intraductal mammary carcinomas and comedocarcinomas and in noninfiltrating bronchial carcinoma. This discontinuous pattern seems to correspond to a potential neoplastic invasive process. In mammary benign lesions there is an inconstant and variable pericellular labeling around epithelial cells. This pericellular FN is never found in severe dysplastic and malignant lesions of the breast. The disappearance of this pericellular and basement membrane FN could be of practical interest in the detection of early malignant lesions. In benign connective proliferations FN is localized principally on cellular surfaces and vascular basement membranes. In sarcomas, there is a loss of the regular pericellular labeling around sarcomatous cells. These observations correlate well with cellular culture findings. Loss of pericellular FN seems to be related to malignant transformation. Detection of FN would be of diagnostic value in pseudo-tumoral or inflammatory lesions of the soft tissues. In the normal skin, FN is present in papillary dermis principally. In the skin of diabetic patients, there is an increase of FN in the superficial dermis and a frequent labeling of epidermis basement membrane. Thickened vascular basement membranes are also labeled by anti-FN serum. FN seems to be involved in the diabetic microangiopathy. In sclerodermic processes, there are qualitative and quantitative modifications of the distribution of cutaneous FN. FN is abundant in the lower dermis, especially in active fibrotic lesions. In the renal parenchyma, FN is found in the mesangium of glomeruli and in vascular basement membranes of the interstitium. FN is a good marker of the mesangium in numerous proliferative glomerulonephritis. FN is also linked to fibrin in thrombotic microangiopathy, epithelial crescents and fibrinoid necrosis. FN is not found in amyloid deposits. (21 Refs) (In FRE)

23. SCANNING ELECTRON MICROSCOPIC FEATURES OF HUMAN URINARY BLADDER CANCER.

Jacobs JB, Cohen SM, Farrow GM, Friedell GH

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Cancer; 48(6):1399-1409 1981

Biopsies from 17 cases of transitional cell carcinoma of the urinary bladder were examined by scanning electron microscopy to evaluate surface features of the luminal cells. Pleomorphic microvilli were observed on cells in all cases regardless of tumor grade. Several types of cell surface patterns were observed but variability in appearance increased with grade as did the degree of pleomorphism of the microvilli. Pleomorphic microvilli were not observed in biopsies from two patients without bladder lesions. (Author abstract) (24 Refs)

24. THE HYALINE INCLUSIONS FOUND IN MENINGIOMA (MEETING ABSTRACT).

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Program and Abstracts of the 22nd Workshop on Selected Topics of the Japanese Society of Neuropathology held in Fukuoka, Japan, 9-11 May, 1981. The Japanese Society of Neuropathology, Kyoto 602, Japan, 136 pp., 1981.

Two patients with meningioma accompanied by many hyaline inclusions were seen. The hyaline inclusions were found inside and outside of the cells. They were PAS positive and stained with colloidal iron, and were considered, from other histochemical findings, to be composed mainly of mucoproteinaceous substances. Electron microscopy showed that these inclusions were covered with a cell membrane having microvillus-like cell projections, and were situated within and between the cells. The inclusions were various in shape but were mainly comprised of highly electron dense granules, vesicles and a membranous substance with a stratified structure. These meningiomas had well-developed desmosomes, interdigitated cell membranes and tonofilaments. In addition to these general findings many microvillus-like projections of the cell membrane were found in the region not connected to the inclusions. Such microvillus-like projections of the cell membrane have hardly ever been seen before in meningioma. These findings suggest that the meningioma cells rarely can differentiate to secretory cells. The morphological development of these inclusions was examined in comparison with other meningiomas including myxomatous meningioma. (no Refs) (In JPN)

25. PLASMA MEMBRANE AS A SENSITIVE TARGET IN RADIATION-INDUCED CELL INJURY AND DEATH: AN ULTRASTRUCTURAL STUDY.

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Int J Radiat Biol; 40(3):305-311 1981

Damage to the plasma membrane of three different human cell types (lymphocyte, cultured Burkitt's lymphoma, and cultured bronchogenic carcinoma) in vitro as a result of sublethal x-irradiation is described. Peripheral blood lymphocytes irradiated with a dose of 50 rad exhibited a localized protrusion of the plasma membrane 15 min after irradiation. The cytoplasm retained its original contour. Many membranous vesicles were found within the protrusion of the plasma membrane. Relative proportion of cells exhibiting different types of morphological damage did not change significantly during the first 3 hr after irradiation. In lymphocytes irradiated with 100 and 500 rad, the plasma membrane protrusions at 15 min post-irradiation were on a larger area of the cell surface and extended farther out into the external milieu. Burkitt's lymphoma (Raji) cells irradiated with 50, 100, or 500 rad did not exhibit protrusion of the plasma membrane; only fenestrations of the plasma membrane were observed. Change in relative proportion of cells exhibiting damage to the plasma membrane during 3 hr post-irradiation was similar to that observed in normal hum an peripheral blood lymphocytes. The bronchogenic carcinoma (AT-264) cells irradiated with 50, 100, and 150 rad exhibited extensive localized protrusions of the plasma membrane 15 min after irradiation. In samples of cells irradiated with 50 and 100 rad, the number of cells exhibiting morphological damage did not change significantly during 3 hr post-irradiation. Results suggest that the plasma membrane of mammalian cells may be one of the primary sensitive targets where radiation-induced injury first occurs. (15 Refs)

26. ALTERATIONS IN SURFACE ULTRASTRUCTURE AND ANIONIC SITES OF RAT DIMETHYLHYDRAZINE-INDUCED INTESTINAL TUMORS.

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Virchows Arch [Cell Pathol]; 37(2):207-216 1981

The relative roles of cell surface shedding and electronegative charge as determinants of metastatic capacity were studied in experimentally produced intestinal tumors. The ultrastructural organization and distribution of anionic sites on the luminal plasma membrane surface components were examined in small intestinal and colonic tumors induced in male Sprague-Dawley rats with 1,2-dimethylhydrazine. The overall distribution of negatively charged groups was demonstrated with ruthenium red staining. Compared to normal epithelial cells, neoplastic cells revealed evidence of decreased cell surface shedding as manifested by decreased numbers of membrane-bound bodies, and an increased quantity of glycocalyx. Malignant cell surfaces were directly exposed to the intestinal lumen as a result of losing the enteric surface coat covering. The exposed microvilli appeared damaged with shortening and blunting. The glycocalyx and surface coat both reacted strongly with ruthenium red indicating the presence of anionic sites. As a result of surface coat loss, the malignant cell surface components revealed an overall decrease in net negative charge. These alterations in cell surface component ultrastructure and electronegative charge appear to be consistent with the low capacity for chemically induced rat intestinal tumors to metastasize. (Author abstract) (15 Refs)

MEMBRANE RECEPTOR/ BINDING SITES

27. GLUCOCORTICOIDS AND BENZO(A)PYRENE HAVE OPPOSING EFFECTS ON EGF RECEPTOR BINDING.

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Nature; 293(5831):404-406 1981

Epidermal growth factor (EGF)-receptor binding was used as a marker for membrane effects in C3H10T1/2 mouse embryo fibroblasts exposed to the potent glucocorticoid fluocinolone acetonide (FA), and to the carcinogens benzo(a)pyrene (BP), and 12-O-tetradecanoyl-phorbol-13-acetate (TPA). FA alone produced a 1.9x stimulation of EGF-receptor binding over that observed in control cultures. The stimulation was apparent within 1 hr and reached a max by 24 hr. FA pretreatment (2 x 10(-7) M) protected the cells from the approx 50% inhibition of EGF-binding induced by BP (2 x 10(-6) M) or TPA (10(-9) M). Results suggested that glucocorticoids inhibit carcinogenesis by inducing cell membrane changes that are reciprocal to those induced by carcinogens and tumor promotors. (22 Refs)

28. COLLAGEN CELL ATTACHMENT PROTEIN FROM RAT HEPATOMA CELLS.

Dickey WD, Seals CM, Klebe RJ
Div. Immunology and Allergy, Dept. Pediatrics, Univ. Texas Medical Branch, Galveston, TX, 77550
Cancer Res; 41(10):4027-4030 1981

This paper describes the isolation and partial characterization of a collagen cell attachment protein from the spent culture medium of rat hepatoma cells. When compared with serum fibronectin, this attachment protein differed in several biochemical parameters. The hepatoma attachment protein was partially purified by adsorbing and eluting from an inorganic gel, magnesium oxide. Cell adhesive activity was routinely recovered at levels of 10 to 30%, and a 2,000-fold purification was attained. The hepatoma attachment protein was shown to be sensitive to trypsin and chymotrypsin, to be heat inactivated at 61 C, to have a mol wt of 58,000, to have an isoelectric point of 4.1, to show an electrophoretic mobility on cellulose acetate of approx one-half that of fibronectin, and not to cross-react with antifibronectin antisera. (Author abstract) (27 Refs)

29. EFFECTS OF LECTINS ON CAMP PRODUCTION AND STEROIDOGENESIS IN CULTURED ADRENAL TUMOR CELLS.

Lifrak E, Chen CC, Wishnow RM
Veterans Admin. Medical Center, Long Beach, CA, 90822
Exp Cell Res; 135(1):167-177 1981

The plant lectins, concanavalin A (conA), wheat germ agglutinin (WGA), and phytohemagglutinin (PHA) stimulate steroidogenesis in cultured adrenal tumor cells. ConA max stimulated steroidogenesis at 100 ug/ml following an approx 4 hr lag phase. ConA stimulation was completely inhibited by alpha-methyl-D-mannopyranoside and the WGA effect was prevented by N-acetyl-D-glucosamine. It was also found that conA alone did not cause a measurable increase in either intra- or extracellular cyclic AMP (cAMP) production. In addition, conA when added simultaneously with ACTH doubled the intra- and extracellular cAMP production over controls treated with ACTH alone. This enhancement effect was dose dependent. When Y-1 cells were preincubated with conA and then treated with either ACTH or cholera enterotoxin (CT) there was a dose- and time-dependent inhibition of induced cAMP production. In the case of CT, the inhibitory effect occurred even with simultaneous addition of conA and CT. This effect was reversed by addition of both alpha-methyl-D-mannopyranoside and washing with Eagle's minimal essential medium (MEM) 1 hr after CT had bound to its receptor. This reversal was not apparent for the inhibitory effect of conA on ACTH-induced cAMP production which occurred after 2 h of preincubation with conA. These results demonstrate that conA, as well as the other plant lectins, interact with specific membrane receptors to reversibly stimulate steroid production as well as enhancing or inhibiting ligand-induced cAMP production in cultured adrenal tumor cells. (Author abstract) (38 Refs)

30. CATECHOLAMINE-INDUCED DESENSITIZATION OF ADENYLATE CYCLASE IN RAT GLIOMA C6 CELLS. EVIDENCE FOR A SPECIFIC UNCOUPLING OF BETA-ADRENERGIC RECEPTORS FROM A FUNCTIONAL REGULATORY COMPONENT OF ADENYLATE CYCLASE.

Fishman PH, Mallorga P, Tallman JF
Building 10, Room 3-D-03, NIH, Bethesda, MD, 20205
Mol Pharmacol; 20(2):310-318 1981

C6 low passage (C6LP) cells accumulated high levels of cyclic AMP (3-4 nanomoles/mg of protein) when exposed to isoproterenol (ISO), but C6 high passage (C6HP) cells did not. Membranes of cells desensitized by prolonged exposure to ISO seemed to have lower affinity to the agonist. Dose and time dependence, effect of phosphodiesterase, and loss of beta-adrenergic receptors were examined. Adenylate cyclase activity in membranes and the relationship to NaF-stimulated activi-

ty were studied. Reaction of the different cell types to cholera toxin, activation of cyclase, and roles of NAD and phosphodiesterase inhibitor were examined. The large accumulation of cyclic AMP in C6LP cells induced by beta-agonists does not appear to mediate the subsequent desensitization process. Initial stages of catecholamine-induced desensitization in C6LP and C6HP cells seem to represent a specific uncoupling of beta-adrenergic receptor from a functional regulatory component of adenylate cyclase. (35 Refs)

TRANSPORT STUDIES

31. INTERACTION OF 2-AMINOBICYCLO[3.2.1]OCTANE-2-CARBOXYLIC ACID WITH THE AMINO ACID TRANSPORT SYSTEMS OF THE SARCOMA 37 MURINE ASCITES TUMOR CELL.

Elliott MS, Matthews RH, Minton JP, Zand R
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Biochemistry; 20(18):5105-5108 1981

The relatively broad and overlapping specificities of amino acid transport systems have made the synthesis of analogues specific to single transport systems desirable. The analogue in general use as a specific substrate for transport system L has been 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH). The affinity of BCH for the binding site of system L has been snown to be less than that of the natural substrate, leucine. Earlier studies from this laboratory suggested that higher homologues in a series could have greater affinity for system L. A higher homologue of BCH, 2-aminobicyclo[3.2.1]octane-2-carboxylic ac id (ABOCA), has been synthesized and studied as a substrate and competitor for amino acid transport systems of the sarcoma 37 (S37) ascites cell. ABOCA inhibited the transport system dominant in the low concentration region for histidine uptake (system L) but had no effect on the uptake of labeled N-methyl-alpha-aminoisobutyric acid (MeAIB). MeAIB had no effect on labeled ABOCA uptake in S37 cells. ABOCA inhibited the uptakes of labeled leucine and labeled BCH competitively. Leucine, histidine, and BCH inhibited the uptake of labeled ABOCA competitively. Typical L system substrates demonstrated exchange effects with labeled ABOCA. The b isomer of ABOCA demonstrated slightly greater affinity for system L than did the a isomer. We conclude that ABOCA is an analogue restricted to interaction with amino acid transport system L, that it has greater affinity for system L than does BCH, and that its selection for system L is determined principally by an apolar interaction with steric considerations secondary. (Author abstract) (18 Refs)

32. IMPROVED METHOTREXATE THERAPY OF MURINE TUMORS OBTAINED BY PROBENECID-MEDIATED PHARMACOLOGICAL MODULATION AT THE LEVEL OF MEMBRANE TRANSPORT.

Sirotnak FM, Moccio DM, Hancock CH, Young CW
Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
Cancer Res; 41(10):3944-3949 1981

Our earlier studies showed that both carrier-mediated influx and efflux of [3H]methotrexate in murine tumor cells was inhibited by probenecid. The concentration of probenecid required for inhibition of influx was 10-fold greater than that required to inhibit efflux. As a consequence, the intracellular exchangeable level of [3H]methotrexate at steady state was markedly increased. Since these effects were observed at concentrations of probenecid which were pharmacologically achievable, studies were initiated in mice to evaluate the adjuvant use of this agent during therapy with folate analogs. The simultaneous administration of probenecid (125 mg/kg sc) with methotrexate (9 to 18 mg/kg sc) on a schedule of every 2 days for five doses to tumor-bearing mice resulted in an increased antitumor effect over that obtained with methotrexate alone. Against the L1210 leukemia, greater than 213% increased life span was obtained compared to 152% increased life span with methotrexate alone. Against the Sarcoma 180 ascites tumor, greater than 179% increased life span was obtained compared to 82% with methotrexate alone. Long-term survivors (10 to 20%) were obtained only with methotrexate plus probenecid. Data on plasma levels of [3H]methotrexate following this schedule of administration showed a 2-fold higher initial level in probenecid-treated mice versus mice given [3H]methotrexate alone, a cross-over in levels in these mice 2 to 3 hr later, and a 2-fold lower level sustained for at least 24 hr. Initial rate and max level of accumulation in prob enecid-treated mice were reduced 2-fold in liver, kidney, bone marrow, and small intestine. Otherwise, overall persistence of [3H]methotrexate in these tissues was minimally affected. In Sarcoma 180 cells, the initial rate but not the max level of [3H]methotrexate accumulation was reduced 2-fold by probenecid. However, the overall persistence of [3H]methotrexate in these cells was increased 2-fold with probenecid. The effect on plasma and tissue levels of [3H]methotrexate was dependent on the dosage of probenecid but did not depend on the dosage of the antifolate itself, at least within the dosage range used. These results show that the effects of probenecid on methotrexate transport, documented for tumor cells in in vitro studies were favorably and selectively expressed in these cells at a pharmacological level and as such may have clinical applications. (Author abstract) (22 Refs)

33. FACILITATED TRANSPORT OF 6-MERCAPTOPURINE AND 6-THIOGUANINE AND NON-MEDIATED PERMEATION OF 8-AZAGUANINE IN NOVIKOFF RAT HEPATOMA CELLS AND RELATIONSHIP TO INTRACELLULAR PHOSPHORIBOSYLATION.

Plagemann PG, Marz R, Wohlhueter RM, Graff JC, Zylka JM
Dept. Microbiology, Univ. Minnesota, Medical Sch., Minneapolis, MN, 55455
Biochim Biophys Acta; 647(1):49-62 1981

The compound 6-Mercaptopurine and 6-thioguanine strongly inhibited the zero-trans entry of hypoxanthine into Novikoff rat hepatoma cells which lacked hypoxanthine/guanine phosphoribosyltransferase, whereas 8-azaguanine had no significant effect. 6-Mercaptopurine was transported by the hypoxanthine carrier with about the same efficiency as its natural substrates (Michaelis-Menten constant = 372 +-23 uM, max velocity = 30 +-0.7 pmol/ul cell H2O per s). 8-Azaguanine entry into the cells, on the other hand, showed no sign of saturability and was not significantly affected by substrates of the hypoxanthine/guanine carrier. The rate of entry of 8-azaguanine at 10-100 uM amounted to only about 5% of that of hypoxanthine transport and was related to its lipid solubility in the same manner as observed for various substances whose permeation through the plasma membrane is believed to be non-mediated. Only the non-ionized form of 8-azaguanine (pKa = 6.6) permeated the cell membrane. Studies with wild type Novikoff cells showed that permeation into the cell was the main rate-determining step in the conversion of extracellular 8-azaguan-

ine to intracellular aza-GTP and its incorporation into nucleic acids. In contrast, 6-mercaptopurine was rapidly transported into cells and phosphoribosylated; the main rate-determining step in its incorporation into nucleic acids was the further conversion of 6-mercaptopurine riboside 5'-monophosphate. (Author abstract) (35 Refs)

34. GLIAL MEMBRANE POTENTIALS AND THEIR RELATIONSHIP TO [K +]O IN MAN AND GUINEA PIG. A COMPARATIVE STUDY OF INTRACELLULARLY MARKED NORMAL, REACTIVE, AND NEOPLASTIC GLIA.

Picker S, Pieper CF, Goldring S
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J Neurosurg; 55(3):347-363 1981

Glial cells were studied in vitro in physiologically viable brain slices prepared from tissues obtained during operations for focal epilepsy and brain tumors. The results from human normal and reactive cerebral cortex and glial tumors were compared with those obtained in guinea pig cortex. The resting membrane potential (RMP) or the relationship of RMP to changes in extracellular potassium concentration ([K +]o) were recorded, and the cell was injected iontophoretically with horseradish peroxidase (HRP) for later visualization and correlation with the physiological data. In normal cortex, the marked cells were protoplasmic astrocytes; in reactive cortex, fibrous astrocytes with abnormal processes predominated. The majority of neoplastic glia resembled the reactive astrocytes, except that they showed a pleomorphism that increased with the degree of malignancy. The mean RMP's of glia from normal human and guinea pig cortex, human reactive cortex, and well differentiated astrocytomas were comparable, about-70 mV. In the less differentiated gliomas, the mean RMP's were lower, with the glioblastoma multiforme having the lowest value,-40 +-7 mV. When the [K +]o was manipulated over a range of 2 to 40 mM/liter, the mean slope of RMP plotted against [K +]o indicated that normal human and guinea pig glia were exclusively permeable to K + between [K +]o of 4 to 40 mM/liter. In contrast, the mean slope of reactive glia at these [K +]o's was less, suggesting a permeability to one or more additional ions. Below 4 mM/liter, the slopes of both normal and reactive glia showed changes in RMP smaller than the values expected of cells that are permeable only to K + . Like the reactive glia, the slopes derived for neoplastic cells did not indicate an an exclusive permeability to K + , and some cells from the glioblastoma multiforme showed no change in RMP when [K +]o was varied. (Author abstract) (65 Refs)

35. EFFECT OF GAMMA RADIATION ON THE TRANSPORT OF SPIN-LABELED COMPOUNDS ACROSS THE ERYTHROCYTE MEMBRANE.

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Radiat Environ Biophys; 19(4):275-285 1981

Transport of spin-labeled compounds into bovine RBC was studied. The compounds used included 2,2,6,-tetramethylpiperidine-1-oxyl (TEMPO), 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL), TEMPOL-choline, and 3-carboxy-2,2,6,6-tetramethylpyrrolidine-1-oxyl. Irradiation of the RBC in the dose range of 2-50 kilorad resulted in a regular dose-dependent increase in the reduction r ates of TEMPO-choline (a cation), and TEMPO (a hydrophobic non-electrolyte), and nonregular changes in the reduction rate of TEMPOL (a hydrophilic non-electrolyte). The permeation constant for TEMPO-choline showed a non-regular response to radiation similar to the response of other RBC parameters. The effects of the radiation on the transport of various solutes can be used as a means of distinguishing between different channels of membrane transport. (19 Refs)

MEMBRANE BIOGENESIS AND METABOLISM

36. BIOSYNTHESIS AND MATURATION OF HLA-DR ANTIGENS IN VIVO.

Owen MJ, Kissonerghis AM, Lodish HF, Crumpton MJ
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J Biol Chem; 256(17):8987-8993 1981

The biosynthesis of the HLA-DR antigens was studied in the B lymphoblastoid cell line BRI 8. Three chains, of mol wt 33,000(alpha), 31,000 (p31), and 26,000 (beta) were detected intracellularly in pulse-labeled cells by immunoprecipitation with anti-(HLA-DR) sera. The alpha and beta chains were inserted asymmetrically into the rough endoplasmic reticulum as transmembrane polypeptides with the majority of the polypeptide chains oriented in the lumen. At this stage, both chains carried 'high mannose' oligosaccharide units which were processed to the complex form during subsequent intracellular transport to the cell surface. The Mr = 31,000 polypeptide was also glycosylated but was structurally distinct from the alpha chain and was probably oriented differently in the lipid bilayer, with a much greater proportion of its polypeptide chain exposed in the cytoplasm. It was not, therefore, a precursor of the alpha chain. The mature HLA-DR antigens at the plasma membrane comprised polypeptides of Mr = 34,000 and 28,000. These chains corresponded to the processed alpha and beta chains. Although the Mr = 31,000 component was only detected intracellularly, it was not ruled out that some or all of it may have been processed and exposed on the cell surface with an apparent mol wt indistinguishable from that of the alpha chain. (Author abstract) (32 Refs)

37. ACTIVATION OF THE METABOLISM OF THE FATTY ACYL GROUP IN GRANULOCYTE PHOSPHOLIPIDS BY PHORBOL MYRISTATE ACETATE.

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Biochim Biophys Acta; 665(3):491-497 1981

Phorbol myristate acetate is known to reproduce the stimulated oxidative activities characteristic of phagocytosis and its initial action is on the cell membrane. In the present study the effect of phorbol myristate acetate on the metabolism of the fatty acyl groups of granulocyte phospholipids was examined and compared with that of phagocytic stimuli. Phorbol myristate acetate stimulated the labeling of phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol by [1-14C]palmitic acid but not by [U-14C]glycerol, whereas starch granules selectively increased the labeling of phosphatidylinositol by both radioactive tracers. Labeled palmitic acid was found at both sn-1-and sn-2-positions of phospholipids and more radioactivity was recovered from the 2-position. The radioactivity at both positions was en-

hanced in stimulated cells. These data suggest that phorbol myristate acetate increased palmitic acid incorporation into glycerophospholipids by increasing the acylation of the lyso derivatives and that starch granules enhanced the formation of phosphatidylinositol via de novo synthesis and acylation of the lyso derivative as well. Both phorbol myristate acetate and starch granules selectively augmented the incorporation of [1-14C]arachidonic acid into phosphatidylinositol which exhibited the highest specific radioactivity among the phospholipids in control and in stimulated cells. The possible significance of the increased incorporation of arachidonic acid into phosphatidylinositol is discussed. (Author abstract) (24 Refs)

38. SIALIC ACID METABOLISM IN REGENERATING RAT LIVER.

Kishore GS, Carubelli R
McArdle Lab. for Cancer Res., Univ. Wisconsin, Madison, WI, 53706
Cancer Lett; 13(4):281-289 1981

Sialic acid content and levels of the enzymes responsible for activation, transfer, and hydrolysis of sialic acids in the livers of control, sham-operated, and two-thirds-hepatectomized male rats were studied. At 24 and 48 hr after surgery, the specific activities of cytidine monophosphate-[4,5,6,7,8,9-14C] N-acetylneuraminic acid (CMP-NANA) synthetase and sialyltransferase in regenerating liver, as well as in livers of sham-operated rats, were significantly higher than in control liver tissues removed at surgery. Combined range of values for the two enzymes (in nanomole/mg protein) were 401-452 vs 324 control and 0.47-0.57 vs 0.36 control, respectively. For each enzyme, values for regenerating and sham-operated liver were not significantly different. Membrane-bound neuraminidase activity was 2x control level in regenerating liver at 24 and 48 hr, but not significantly different from control in sham tissue. Cytosolic neuraminidase activity was similar in all tissue samples. Regenerating liver sialic acid content, determined by thiobarbituric acid assay, was lower at 24 but not at 48 hr than in sham or control liver samples; the changes were observed in whole liver homogenate and in the sedimentable fraction but not in the soluble fraction. Results for CMP-NANA synthetase and sialyltransferase did not constitute changes specifically associated with the regenerating process, since similar changes were seen in regenerating and sham-operated tissue. The selective increase in membrane-bound neuraminidase and decrease in sialic acid content in regenerating liver were of particular interest, in light of reduced activity obs erved for other regenerating rat liver acid hydrolases and increases in sialic acid seen in various malignant hepatic tissues. (27 Refs)

CELL ADHESION

39. CELL ADHESION-DEPENDENT DIFFERENCES IN ENDOGENOUS PROTEIN PHOSPHORYLATION ON THE SURFACE OF VARIOUS CELL LINES.

Pfeifle J, Hagmann W, Anderer FA
(c/o Anderer), Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Spemannstrasse 37-39, 7400 Tubingen, W. Germany
Biochim Biophys Acta: 670(2):274-284 1981

Endogenous phosphorylation of intact cells was studied with four mouse, hamster and human cell lines using [gamma-32P]ATP and [gamma-32P]GTP as exogenous substrates. With all four cell lines distinct differences in the phosphoprotein patterns could be demonstrated for cells grown in suspension culture compared to cells grown in monolayers. Two major, apparently ubiquitous phosphoproteins with mol wt of 135,000 (128,000 in HeLa cells) and 105,000, representing up to 60% of total phosphorylation, were phosphorylated only in cells grown in suspension. These phosphoproteins and the kinase(s) were located on the surface of the suspension cells. Evidence showed that phosphorylation was apparently not a true endogenous reaction, that rather it occurred by cell-cell collision, showing exponentially increasing 32P incorporation with increasing cell population density. Phosphorylation of pp135 and pp105 was established with ATP as well as with GTP and was not dependent on cyclic nucleotides cyclic AMP, cyclic GMP and cyclic CMP. The substrate-attached cells of all four cell lines have protein kinases on the cell surface. The lack of pp135 and pp105 phosphorylation may be due to the fact that these phosphoproteins are not expressed at all on the surface of substrate-attached cells or that these phosphoproteins are already fully phosphorylated. (Author abstract) (32 Refs)

40. MOLECULAR PATHOLOGY OF CANCER CELL ADHESIVENESS.

Ishimaru Y, Koga Y, Tokuda S, Hayashi H
Dept. Pathology, Kumamoto Univ. Medical Sch., 2-2-1 Honjo-machi, Kumamoto 860, Japan
Acta Pathol Jpn; 31(4):623-640 1981

A new cell surface-associated adhesive glycoprotein with a mol wt of 70,000 was separated from differentiated rat ascites hepatoma cells forming cell islands in vivo (but not from undifferentiated rat ascites hepatoma cells present as single cells in vivo) and highly purified by chromatography; it was synthesized by the cells and localized on the cell surface. Its synthesis began to rise rapidly and reached its peak in 24 hr cultivation, ie, a 10-fold increase. This substance induced not only aggregation but also adhesiveness of the cells characterized by junctional complexes including tight junctions, desmosomes, and intermediate junctions, closely resembling the frequency and distribution of junctional complexes observed on the above cells islands. Its potency was inhibited specifically by D-mannose and alpha-methyl-D-mannoside; the numbers of the binding sites per cell were calculated as 6 x 10(5). Its activity was concerned with the protein portion of the molecule, and not with the carbohydrate portion. Thus, it seemed reasonable that the adhesive glycoprotein may play a key role in the cell adhesiveness and island formation. In contrast, serum-associated adhesive glycoprotein, separated from normal rat serum, could aggregate the cells but not develop junctional complexes. (Author abstract) (45 Refs)

41. BS I-B4 ISOLECTIN AS A PROBE FOR AN INVESTIGATION OF MEMBRANE ALTERATIONS AND TRANSFORMATION PHENOTYPES OF MOUSE L CELLS.

Stanley WS, Chu EH
Michigan Cancer Foundation, 110 East Warren St., Detroit, MI, 48201
J Cell Sci; 50:79-88 1981

BS I-B4, an alpha-D-galactopyranosyl-binding isolectin from Bandeiraea simplicifolia seeds, was found to interact differently with transformed mouse L cells and non-transformed mouse 3T3 cells. The lectin induces detachment of 3T3 cells but increases adhesiveness and clustering of L cells. However, the induced cell aggregation does not lead to cell fusion. A variant clone of L cells, resistant to BS I-B4, which had lost the capacity for agglutination in the presence of the lectin, was iso-

lated ... fluorescence binding studies of this variant suggest a les... involving alpha and beta-D-galactopyranosyl units on its c... surface structures. Although the variant cells form colonies ... methylcellulose medium, they do not produce tumors, as ... the parental cells, when transplanted in athymic nude mice. ... results demonstrate that alterations in cell membrane ... glycoconjugates play an important role in tumorigenesis of ... cells, but anchorage-independent growth in vitro, as ... of the transformation phenotypes, cannot be correlated ab... with tumorigenicity in vivo. (Author abstract) (20 Refs)

42. EFFECT OF CIS-DICHLORODIAMMINE PLATINUM(II) ON THE AGGLUTINABILITY OF TUMOR AND NORMAL CELLS WITH CONCANAVALIN A AND WHEAT GERM AGGLUTININ.

Bali Prasad S, Sodhi A
(c/o Sodhi) Dept. Zoology, Banaras Hindu Univ., Varanasi 221005, India
Chem Biol Interact; 36(3):355-367 1981

In this paper we report the effect of cis-dichlorodiammine platinum(II) (cis-platin) on the agglutination of normal and transformed lymphocytes with lectins conconavalin A (Con A) and wheat germ agglutinin (WGA) which is a sensitive test to study the expression of cell surface components. Normal splenocytes showed small degree of cell agglutination with Con A and WGA. This cell agglutination of normal splenocytes with these lectins increases significantly after cis-platin treatment at 37 C. The Dalton's lymphoma cells which show a high degree of cell agglutination with Con A and WGA when treated with cis-platin at 37 C results in decrease in the degree of their cell agglutination with these lectins. Agglutination inhibition studies with alpha-methyl-D-glucopyranoside for Con A and N-acetyl-D-glucosamine for WGA shows that this increase and decrease of cell agglutination after platinum treatment of cells is a specific reaction and not non-specific. Incubation of cis-platin treated normal splenocytes with excess of neuraminic acid results in decrease of cell agglutination with both Con A and WGA. cis-platin treatment of the cells results in the release of sialic acid from the surface of the cells. A correlation between the release of sialic acid from the cell surface and cell agglutination is also discussed. (Author abstract) (30 Refs)

OTHER RELATED STUDIES

43. CELL-SPECIFIC DRUG TRANSFER FROM LIPOSOMES BEARING MONOCLONAL ANTIBODIES.

Leserman LD, Machy P, Barbet J
Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France
Nature; 293(5829):226-228 1981

A technique for the covalent coupling of monoclonal antibodies to small sonicated liposomes was used to study specific delivery of methotrexate (MTX) to cellular targets in vitro. Murine IgG2a-kappa monoclonal antibodies (11-4-1) and H40.242.3 with specificity, respectively, for molecular determinants of H-2K-kappa and H-2 I-E-kappa, were coupled to liposomes containing MTX and carboxyfluorescein (CF). Aliquots of the liposome preparations were incubated with lipopolysaccharide-induced blast cells from CBA(H-2-kappa) and C57BL/6(B6)(H-2b) spleens. After 3 hr, the amount of cell-associated CF was determined by fluorometry. Liposome binding was specific: CBA cells bound many more 11-4-1 liposomes than did B6 cells (29 vs 0.4 picomole cell-associated CF). The binding was inhibited by preincubation of cells with the uncoupled homologous antibody (CBA cell CF decreased to 0.5 picomole in the presence of 11-4-1 free antibody) but not with an irrelevant (B1.1G6) antibody of the same class. Bound liposomes were capable of transferring MTX into the target cells, as indicated by inhibition of 3H-deoxyuridine uptake in CBA cells. Magnitude of the drug effect was not directly related to density of target antigen: cell association of 11-4-1 was approx 2x that of H40.242.3, but the number of 11-4-1-bearing liposomes required for 50% inhibition of 3H-deoxyuridine incorporation was 4x greater. Thus, liposomes bound to the H-2 I-E-kappa determinant were 8x more effective in MTX delivery than those bound to the H-2K-kappa determinant. The fact that the MTX effect was inhibited by the lysosomotropic amine NH4Cl only when carried by liposomes, but not in free form, suggested an endocytic pathway for the liposome-encapsulated drug. (15 Refs)

44. INABILITY OF LIPOSOME ENCAPSULATED 1-BETA-D-ARABINOFURANOSYLCYTOSINE NUCLEOTIDES TO OVERCOME DRUG RESISTANCE IN L1210 CELLS.

Rustum YM, Mayhew E, Szoka F, Campbell J
Dept. Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Inst., 666 Elm St., Buffalo, NY, 14263
Eur J Cancer Clin Oncol; 17(7):809-817 1981

The uptake, metabolism and antitumor activity of 1-beta-D-arabinofuranosylcytosine nucleotides encapsulated in liposomes were investigated in L1210 cells sensitive and resistant to the drug. These studies were carried out in order to determine whether encapsulation of the presumed active component of the drug, namely, arabinofuranosylcytosine 5' triphosphate will convert resistant cells to sensitive. The results indicate that liposome entrapment of Ara-CMP and Ara-CTP did not result in increased delivery of these metabolites into L1210 cells. The amount of nucleotide found intracellularly following an in vitro incubation of encapsulated drugs with L1210 cells did not exceed the level expected from a simple extracellular breakdown of liposome releasing their contents with subsequent uptake and phosphorylation of the released nucleoside. Further evidence for lack of enhancement of drug uptake by liposome encapsulation was obtained when the rate of incorporation of TdR into DNA of L1210/Ara-C was not significantly affected by Ara-CTP encapsulation in liposome. The data presented herein also demonstrated that the in vivo sensitivity of L1210 cells resistant to Ara-C could not be modified by encapsulation of Ara-CTP in liposomes. The results strongly suggest that encapsulation of drugs unmodified or untargeted liposomes will not be able to overcome drug resistance related to transport defect and/or deletion of intracellular activating enzyme(s). (Author abstract) (23 Refs)

45. TWO INDEPENDENT ASPECTS OF TUMOR PROMOTION.

Berenblum I, Armuth V
Weizmann Inst. Science, Rehovot, Israel
Biochim Biophys Acta; 651(1):51-63 1981

The possibility of separate and independent mechanisms of tumor promotion is explored based on a review of evidence for sites of action within the cell. The multi-stage theory of carcinogenesis is presented. Evidence for two types of promoting action is discussed: that operating at the regulator-gene level and that operating at the level of the cell-surface membrane.

Tumor promotion is generally regarded as an epigenetic phenomenon in an initiated cell which permits the phenotypic expression of an altered genotype. Since an influence on the cell's mutation rate or its repair mechanisms would need to operate close in time to initiation, these mechanisms are not widely regarded as likely. Growing evidence suggests that promotion may proceed by the derepression of a neighboring regulator gene brought about by the action of some newly synthesized tumor-specific protein(s). Cell membranes in general and cell-surface membranes in particular have an important role in regulating cellular functions, and a great variety of changes in cell-surface membranes is known to effective in this regard. Lack of cell adhesiveness and of contact inhibition in neoplastic cells have long been recognized as cell-surface phenomena. There is evidence that promoters, particularly phorbol ester promoters, can bind to cell-surface membranes and alter their properties. Induction of plasminogen activator, prostaglandins, enzymes of polyamine biosynthesis, and alterations in cyclic nucleotide levels are known cytoplasmic effects of promoter action. Implications and interrelations of these findings are discussed. (224 Refs)

46. CELL MEMBRANE CHARACTERISTICS AND BIOLOGICAL BEHAVIOR OF VIRUS TRANSFORMED CELLS.

Bauer H
Institut Virologie, Fachbereich Humanmedizin, Justus Liebig-Universitat, Frankfurter Strasse 107, D-6300 Giessen, W. Germany
Klin Wochenschr; 59(17):957-964 1981

The transformation of a normal into a tumor cell is not caused by a single molecular event, but is the consequence of several simultaneous or consecutive molecular processes, which lead to a variety of changes in the structure and the metabolism of the cell. Investigations with the Rous sarcoma virus show that a single gene is primarily responsible for these changes that is coding for a phosphoprotein which, however, is multifunctional. The biochemical and biologic events which initiate and maintain the transformed status of the cell involve mainly the cytoplasmic membrane. At both the outer and the inner surface of the cell membrane dramatic changes occur which influence the cell structure, permeability of the cytoplasmic membrane, and the intracellular metabolic pathways. Most probably, these transformation-associated events are also involved in cell proliferation under physiologic conditions. In the tumor cell, however, they are not further regulated physiologically, with the consequence of an uncontrolled and incessant cell division. (Author abstract) (30 Refs) (In GER)

47. DESTABILIZATION OF PLASMATIC MEMBRANE AS MECHANISM OF TUMOR CELLS PROTECTION AGAINST IMMUNITY.

Volkov EI, Chernavskii DS
P. N. Lebedev Physical Inst., Moscow, USSR
Usp Sovrem Biol; 91(3):419-432 1981

The phenomenon of progressive tumor growth despite the pronounced humoral and cellular immune response are discussed. Tumor protection against immunity can be explained by weak immunogenicity of tumor antigens or by shedding off the tumor antigens or antigen-antibody complex and blocking the receptors of cytotoxic T lymphocytes. The physical mechanisms of these phenomena can be associated with the loss of stability of tumor cell membrane. Development of the solid-carcass liquid-mosaic model of cell membrane provided an explanation for tumor 'escape'. It was concluded that polyvalent surfactants can stabilize c ell membrane and thus enhance the antitumor immunity. (48 Refs) (In RUS)

48. MOBILITY OF SURFACE PROTEINS ON NORMAL RAT MACROPHAGES AND ON A 'MACROPHAGELIKE' RAT TUMOR.

Woda BA, Yguerabide J, Feldman JD
Dept. Anatomic Pathology, City of Hope Natl. Medical Center, Duarte, CA, 91010
J Cell Biol; 90(3):705-710 1981

Peritoneal macrophages endocytosed their histocompatibility antigens (RT1), Fc receptors (FcR), and concanavalin A (Con A) receptors after cross-linking by ligands, but did not cap these membrane proteins. The 323N cell, a 'macrophage like' tumor cell, under identical conditions capped its surface proteins. Experiments measuring fluorescence recovery after photobleaching showed that the mobile fraction of RT1 was significantly greater in 323N cells than in normal peritoneal macrophages. Presumably, the membrane proteins of 323N are not as tethered to the cytoskeleton, or, if so, are in a nexus that is not the same as that which occurs between membrane proteins of normal macrophages and the cytoskeleton. The mobility of RT1 on normal lymphocytes was also different from that of macrophages. These observations suggest that the movement of membrane molecules is determined by cell type and is regulated by the cytoskeleton which varies in structure and function from cell type to cell type. (Author abstract) (18 Refs)

49. EFFECTS OF HYPERTHERMIA ON CELL SURFACE CHARGE AND CELL SURVIVAL IN MASTOCYTOMA CELLS.

Sato C, Nakayama T, Kojima K, Nishimoto Y, Nakamura W
Lab. Experimental Radiology, Aichi Cancer Center Res. Inst., Chikusa-ku, Nagoya 464, Japan
Cancer Res; 41(10):4107-4110 1981

Changes in the structure of the surface of mastocytoma cells were induced by hyperthermia and were investigated by means of cell electrophoresis. A decrease in the cell electrophoretic mobility was detected as early as 15 min after treatment at 42 C and progressed more rapidly under hypoxic conditions than under oxic conditions. Subsequent recovery of electrophoretic mobility at 37 C was dependent on the length of heat treatment and oxygenation. The surviving fraction of cells detected by their colony-forming ability and the fraction of electrophoretically recovered cells 24 hr after exposure to hyperthermia showed a good statistical correlation. It was suggested that the mechanism of electrophoretic mobility reduction by heating was the vertical translocation of hyaluronidase-sensitive charge from the peripheral layer into a deeper layer by combined use of specific enzymes and stepwise different ionic strengths. These results suggest the importance of irreparable changes of membrane conformation in the loss of colony-forming ability of heated tumor cells. (Author abstract) (25 Refs)

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. ANALYSIS OF LECTIN-SPECIFIC CELL SURFACE GLYCOPROTEIN ON NEUROBLASTOMA CELLS.

Maher P, Molday RS
Dept. Biology, Univ. California at San Diego, La Jolla, CA, 92093
Biochim Biophys Acta; 647(2):259-269 1981

The binding sites for the lectins wheat germ agglutinin, Ricinus communis agglutinin and concanavalin A on mouse neuroblastoma cell membranes were identified using SDS-gel electrophoresis in combination with fluorescent lectins. Ricinus communis agglutinin and wheat germ agglutinin were found to bind almost exclusively to a single polypeptide with an appar-

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ent mol wt of 30,000. Concanavalin A labeled over 20 different polypeptides, most with mol wt greater than 50,000. However, when the neuroblastoma cells were treated with concanavalin A so as to internalize all the concanavalin A binding sites visible at the level of the fluorescent microscope and the purified plasma membranes analyzed for their concanavalin A binding polypeptides, only four of the 20 glycopolypeptides were missing or significantly reduced in amount. Thus, these four high mol wt concanavalin A-binding polypeptides appear to be the major cell surface receptors for concanavalin A. Binding studies with iodinated concanavalin A indicated that these polypeptides represented the high affinity concanavalin A binding sites (Kd = 2 x 10(-7) M). Low affinity concanavalin A binding sites were present on the cell surface after internalization of high affinity concanavalin A binding sites. (Author abstract) (36 Refs)

2. BIOCHEMICAL CHARACTERIZATION OF AN ANTIGEN ASSOCIATED WITH ACUTE LYMPHOBLASTIC LEUKEMIA AND LYMPHOCYTE PRECURSORS.

Newman RA, Sutherland DR, Greaves MF
Imperial Cancer Res. Fund Lab., P.O. Box 123, Lincoln's Inn Field, London WC2A 3 PX, England
Haematol Blood Transfus; 26:326-328 1981

An antigen (ALLA) occurring on the surface membrane of cells from patients with common acute lymphoblastic leukemia (ALL) was previously identified. ALLA was also found in some cases of undifferentiated leukemias and chronic myeloid leukemia and in a small proportion of normal bone marrow cells. ALLA from the cell line Nalm-1 was characterized biochemically. ALLA is a glycosylated polypeptide of approx 100,000 daltons. No intrachain disulfide linkages are present. ALLA shows 50% binding with lentil lectin, but 100% binding with Ricinis communis lectin. Lectin binding and nonbinding forms form identical homogeneous spots in two-dimensional isoelectric focusing with a pI of 5.1-5.5. Neuraminidase treatment of both forms causes a shift of approx 0.6 towards the basic end of the gel. Treatment with tunicamycin and glycosidases indicate that up to 25% of ALLA mol wt may be carbohydrate, but carbohydrate is not involved in the antigenic site. ALLA is apparently not integral to the cell membrane, but is closely associated with it. Presence of ALLA in some normal bone marrow cells suggests the antigen is a normal product reflecting target cells for malignant transformation and/or the stage of maturation arrest of ALL cells. (11 Refs)

3. GRANULOPOIETIC DIFFERENTIATION IN AML BLASTS IN CULTURE.

Marie JP, Izaguirre CA, Civin CI, Mirro J, McCulloch EA
(c/o McCulloch), Ontario Cancer Inst., 500 Sherbourne St., Toronto, Ontario, M4X 1K9, Canada
Blood; 58(4):670-674 1981

A newly described monoclonal antibody detected an antigen (My-1) that is expressed on the cell surface during normal granulopoietic differentiation. The kinetics of expression of My-1 and NA SD chloracetate esterase were studied during the formation in culture of blast colonies and normal granulopoietic colonies. My-1-positive cells increased during blast colony formation in six of nine experiements, while NASD esterase-positive cells decreased. In contrast, in normal granulopoiesis in culture both markers increased coherently. We suggest that components of granulopoietic differentiation programs are expressed abnormally during blast colony formation in culture. (Author abstract) (19 Refs)

4. CORRELATION OF GLYCOSPHINGOLIPIDS AND SIALIC ACID IN YAC-1 LYMPHOMA VARIANTS WITH THEIR SENSITIVITY TO NATURAL KILLER-CELL-MEDIATED LYSIS.

Yogeeswaran G, Gronberg A, Hansson M, Dalianis T, Kiessling R, Welsh RM
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Int J Cancer; 28(4):517-526 1981

Sialoglycoconjugates and glycosphingolipids were quantitated in a series of variants derived from the YAC-1 lymphoma, known to be highly sensitive to natural killer (NK)-cell-mediated lysis. The variants, which had widely diverging sensitivities to NK cells, were obtained by a number of methods, including selection in the presence of NK cells, antibody to H-2, or antibody to the murine leukemia-virus-induced antigen, and by fusion of sensitive cells with an NK-resistant cell line, A9HT. The sensitivities of these cells to NK-cell-mediated lysis did not correlate with their sensitivities to anti-H-2a cytotoxic T cells. While no correlation could be made between the NK-sensitivity of these variants and their total cellular sialic acid, a statistically significant inverse correlation was observed between the levels of percentage neuraminidase releasable surface sialic acid of total labeled sialyl components and sensitivity to NK cells. This correlation with cell surface sialic acid was observed with either endogenous or lymphocytic choriomeningitis virus-induced activated NK cells as effectors. Neuraminidase treatment of insensitive target cells caused a moderate increase in sensitivity but failed to render the resistant targets as sersitive as YAC-1. Analysis of glycosphingolipids among the variants revealed a strong positive correlation between the total cell neutral glycolipid with chromatographic migration of asialo-GM2 and sensitivity to endogenous or activated NK-cell-mediated lysis. Significant correlations were not found with any other neutral glycolipids. However, ganglioside homologues with chromatographic mobility of GM1, GD1a, GD1b, and GT also showed a positive correlation with both endogenous and activated NK-cell-mediated lysis. The ratio of asialo-GM2 to GM2 had a highly significant positive correlation with sensitivity. These correlative results suggest that asialo-GM2 and certain gangliosides could be involved in binding or lytic events in NK cell:target cell interactions, and that high levels of sialic acid and sialylation on the cell surface may inhibit and/or modify such interactions. Further studies with these YAC variants should be useful for examining the biochemical basis of target cell-effector cell interactions in the NK system. (Author abstract) (41 Refs)

5. LOSS OF MUCOSAL SECRETORY COMPONENTS AND CHANGES IN GLYCOCONJUGATE COMPOSITION OF RAT COLONIC TUMORS (MEETING ABSTRACT).

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Gastroenterology; 80(5,part2):1183 1981

The aim of this study was to characterize the changes in carbohydrate composition of epithelial cell membranes (ECM) and goblet cell mucus (GCM) in transitional mucosa and benign and malignant colonic neoplasms. Colonic tumors were induced in 16 rats with 16 weekly injections of dimethylhydrazine. All animals developed neoplasms within 24 wk. Fluorescein isothiocyanate (FITC) conjugated lectins were then used to stain tissue sections from normal rats and those with tumors. Control sections were incubated with 0.3 M of the binding inhibitor to confirm specificity. Binding to N-acetyl glucosamine

was measured using Wheat germ agglutinin (WGA), mannose and glucose with Concanavalin A (Con A), galactose with Ricinus communis (RCA1), sialic acid with Limulus polyphemus (LPA) and fucose with Ulex europeus (UEA1). No specific changes in lectin binding could be consistently demonstrated to ECM or to transitional mucosa. The data show that GCM and its secretory elements were absent in colonic adenocarcinoma. In benign adenomas lectin staining of GCM GlcNac, Man, Glc, Gal and Fuc residues was diminished while that of NeuNAc was unchanged; the decrease in GCM appeared to be greatest in the upper crypt. Thus alterations in GCM biosynthesis appear to correlate specifically with the degree of tumor malignancy, whereas in transitional mucosa goblet cell glycoconjugates appear normal. (no Refs)

6. SUBCELLULAR LOCALIZATION AND ISOLATION OF GAMMA-GLUTAMYLTRANSFERASE FROM RAT HEPATOMA CELLS.

Ding JL, Smith GD, Peters TJ
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Biochim Biophys Acta; 661(2):191-198 1981

Cultured rat hepatoma cells were homogenized and subjected to subcellular fractionation by analytical sucrose density centrifugation to determine the localization of gamma-glutamyltransferase ((5-glutamyl)-peptide:amino-acid 5-glutamyltransferase, EC 2.3.2.2). The activity was exclusively localized to the plasma membrane. Diazotized sulfanilic acid was used as a non-penetrant membrane reagent which inactivates ectoenzymes. With both intact and sonicated cells, only 70-75% inhibition of gamma-glutamyltransferase activity was observed. At least 12% of the total cell complement of gamma-glutamyltransferase activity is highly resistant to inactivation by diazotized sulfanilic acid even after Triton X-100 solubilization. The enzyme was purified from hepatoma cells and its properties compared with enzyme from normal liver. Apart from the striking increase in Vapp there were only minor differences between the enzymes from the two sources. In contrast to the complete abolition of transpeptidase activity of the purified hepatoma enzyme by diazotized sulfanilic acid, the hydrolytic activity of this preparation was only slightly inhibited. (Author abstract) (28 Refs)

7. ELECTRON-CYTOCHEMICAL AND LIGHT MICROSCOPIC INVESTIGATION OF MG++-DEPENDENT ATPASE ACTIVITY IN THE EHRLICH ASCITIC TUMOR CELLS OF MICE.

Lu Z, Wang K, Zhang J, Liu K
Dept. Cell Biology, Beijing Inst. Cancer Res., Beijing, People's Republic of China
Acta Anat Sin; 12(2):174-178 1981

The presence of Mg++-dependent ATPase has been reported previously in the cell membrane, nucleus and nucleolus of Ehrlich ascitic tumor cells. The analysis of this enzyme and its distribution in these tumor cells as observed by electron-cytochemical and light microscopic methods are described. The ascitic fluid produced 5-8 days (usually 7 days) after tumor cell inoculation was used. The Mg++-dependent enzyme was found to concentrate on the cell membrane and mitochondria. The enzyme present in the mitochondria was highly sensitive to glutaraldehyde and lost its activity after glutaraldehyde fixation, however, its activity was still retained on the cell membrane after glutaraldehyde fixation, showing strong precipitating reactions with lead ions. Treatment of the basal incubating media with L-cysteine enhanced the activity of the enzyme both in the mitochondria and on the cell membrane but treatment with NaF and p-chloromercuribenzoate inhibited its activity. (6 Refs) (In CHI)

MORPHOLOGICAL STUDIES

8. IN VIVO STUDIES OF ACTIN-CONTAINING CYTOSKELETAL ABNORMALITIES IN NEOPLASTIC CELLS.

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Eur J Cell Biol; 25(1):218-222 1981

The role of cytoskeletal abnormalities in the spread of malignant cells and metastasis is discussed. The possibility that defective control of cell motility is expressed in actin-containing cytoskeletal alterations accompanying malignant tumor invasion is considered. Projections into the basement membrane of carcinomas arising from stratified squamous epithelium have been observed. Wide-necked pseudopods 3 um or more in diameter and projecting into the basement membrane are likely to be significant for tumor invasion. The formation of pseudopodia needs to be accompanied by sufficient changes in cell-to-cell adhesive contacts to allow cells to translocate into the basement membrane. Cell junctional contacts observed in adenocarcinomas and sarcomas are described. The possible involvement of host response in bringing about the cytoskeletal abnormalities and the motile behavior of malignant cells is considered. (57 Refs)

9. COMPARATIVE STUDIES BY SCANNING ELECTRON MICROSCOPY OF THE EFFECT OF RICIN ON THE CELL MEMBRANE OF HEPATOMA CELLS AND ISOLATED HEPATOCYTES.

Creppy EE, Lugnier AA, Beck G, Dirheimer G, Petzinger E, Frimmer M
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Toxicol Eur Res; 3(4):179,181-184 1981

The effect of ricin poisoning on hepatoma tissue culture (HTC) cells and normal hepatocytes was studied by scanning electron microscopy. HTC cells treated with 5 picomole/ml ricin developed numerous cell surface protrusions within 8-12 hr. The protrusions were observed on all treated cells and were produced only by the intact ricin molecule. Concentrations of ricin between 5 and 150 picomole/ml had no effect on the shape of normal hepatocytes, whereas extremely high concentrations (750 picomole/ml) caused membrane alterations similar to those observed on the HTC cells. This effect was seen on only 10-20% of the normal hepatocytes within 8 hr. Two possible mechanisms of action of ricin are suggested. (15 Refs)

10. ELECTRON MICROSCOPIC STUDY OF DISSOCIATION OF RAT ASCITES HEPATOMA CELLS FORMING ISLAND IN VIVO.

Koga Y
First Dept. Pathology, Kumamoto Univ. Medical Sch., Kumamoto, Japan
Kumamoto Med J; 34(2):91-107 1981

Two means of dissociation of rat hepatoma AH136B and AH7974 cells sc proliferated are d scribed. One may be associated with the disruption of junctional complexes, including tight junctions, desmosomes, and intermediate junctions, and the other with the cytoplasmic separation followed by engulfment of junctional complexes into the cytoplasm. (25 Refs)

11. INTERCELLULAR JUNCTIONS IN PRECANCEROUS AND CANCEROUS LESIONS OF THE UTERINE NECK (MEETING ABSTRACT).

Schindler AM, Amaudruz MH, Gabbiani G
Centre de Cytologie et depistage du cancer, Geneva
Schweiz Med Wochen Schr; 111(23):870 1981

In a morphometric study, at the electron microscopic level, we compared the distribution of desmosomes and 'gap junctions' in the following lesions of the cervix: 1) metaplasia, 2) moderate dysplasia, 3) marked dysplasia, 4) carcinoma in situ, and 5) invasive epidermoid carcinoma. The proportion of the cell surface occupied by desmosomes gradually diminishes from metaplasia to invasive carcinoma. Metaplasia and moderate dysplasia are considerably different from carcinoma in situ and invasive carcinoma. Marked dysplasia differs little from either moderate dysplasia or carcinoma in situ. The proportion of 'gap junctions' on the cell surface is significant decreased in all of the groups, compared to the controls. Beginning with moderate dysplasia, these junctions are statistically absent. In conclusion, it can be stated that the proportion of the cell surface occupied by desmosomes gradually decreases from moderate dysplasia to invasive carcinoma. 'Gap junctions' gradually disappear from moderate dysplasia to invasive epidermoid carcinoma. (no Refs) (In FRE)

MEMBRANE RECEPTOR/ BINDING SITES

12. TARGETED KILLING OF CULTURED CELLS BY RECEPTOR-DEPENDENT PHOTOSENSITIZATION.

Mosley ST, Goldstein JL, Brown MS, Falck JR, Anderson RG
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Proc Natl Acad Sci USA; 78(9,part2):5717-5721 1981

This paper describes a method, designated 'receptor-dependent photosensitization', by which the receptor-mediated endocytosis of low density lipoprotein (LDL) can be used to deliver a photosensitizing agent, pyrene, to cultured human and animal cells. The hydrophobic core of LDL is extracted and replaced with pyrene covalently coupled to cholesteryl oleate. This reconstituted LDL enters cells in significant amounts only when the cells express LDL receptors, resulting in the accumulation of pyrene cholesteryl oleate within lysosomes. Subsequent exposure of the cells to UV light leads to cell death. Cells killed by this technique include normal and simian virus 40-transformed human fibroblasts, human A-431 epidermal carcinoma cells, Chinese hamster ovary cells, and mouse L cells, all of which express LDL receptors. Mutant fibroblasts from a patient with homozygous familial hypercholesterolemia, which lack LDL receptors, do not take up significant amounts of the pyrene-containing LDL and are not killed by subsequent exposure to light. The current experiments establish the feasibility of receptor-dependent photosensitization as an efficient and selective method for killing cultured human and animal cells. (Author abstract) (24 Refs)

13. DYNAMICS OF CALMODULIN AND CYCLIC AMP PHOSPHODIESTERASE IN PLASMA MEMBRANES OF RAT LIVERS AND ASCITES HEPATOMAS.

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Biochim Biophys Acta; 677(2):174-183 1981

Plasma membranes from ascites hepatoma cells (AH-7974, AH-130) contained much smaller amounts of calmodulin (about half) and cyclic AMP phosphodiesterase (about one-third) compared to plasma membranes of rat livers. Some calmodulin molecules in liver plasma membranes were released by repeated washing. The 'washed' liver plasma membranes showed the presence of specific binding sites for externally added calmodulin molecules (bovine brain) (N = 140 pmol/mg protein Kd = 7.9 X 10(-8) M). The calmodulin content of AH-7974 plasma membranes was not reduced by repeated washing. The binding of calmodulin to the 'washed' AH-7974 plasma membranes was only of nonspecific nature with negative cooperativity. Plasma membranes (liver and AH-7974) appeared to contain both calmodulin-dependent and calmodulin-independent phosphodiesterase, but the stimulation by externally added Ca++ plus calmodulin was rather small. Externally added calmodulin-dependent phosphodiesterase (bovine brain) was bound more to 'washed' liver plasma membranes than to 'washed' AH-7974 plasma membranes. Newly bound phosphodiesterase appeared to be more sensitive to the stimulation by Ca++ plus calmodulin in 'washed' hepatoma plasma membranes than in 'washed' liver plasma membranes. Preincubation of 'washed' plasma membranes (liver and hepatoma) with calmodulin did not affect the binding of phosphodiesterase, but the sensitivity of phosphodiesterase to the stimulation by Ca++ plus calmodulin in hepatoma plasma membranes was lost. (Author abstract) (21 Refs)

14. GASTRIN RECEPTORS IN HUMAN COLON CARCINOMA (MEETING ABSTRACT).

Rae-Venter B, Townsend CM, Thompson JC, Simon PM
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Gastroenterology; 80(5,part2):1256 1981

The purpose of this study was to determine whether normal or neoplastic human colons possess gastrin (GR). Partially purified plasma membranes were prepared from 8 human colon carcinoma and 3 normal colon biopsy specimens by discontinuous sucrose density gradient cetrifugation. The particulate material that collected at the 40-50% interface was used for evaluation of receptor binding. 125I-labeled synthetic human gastrin (*G-17) was prepared using EnzymoBeads (BioRad). For assays of specific gastrin receptors, 500 mg of membrane protein was incubated with 100 pM *G-17 for 2 hr at 20 C. Nonspecific binding was determined from a parallel assay in which the membranes were preincubated for 20 min at 20 C with 100 nM unlabeled G-17. Seven out of 8 of the tumor specimens had GR. The binding of *G-17 was inhibited by preincubation of the membrane with homologous peptide (100 nM), pentagastrin, caerulein and CCK-8 and 15-Leu-G-17. However, 15-Leu-G-17 was significantly less effective than 15-Met-G-17 in com-

peting for *G-17 binding. Secretin and glucagon were noncompetitive inhibitors of *G-17 binding. The concentration of GR in 3 specimens of normal tissue ranged from 0.4 to 2.5 fmoles/mg membrane protein. Human neoplasms originating in those tissues normally responsive to the trophic effects of gastrin have receptors for gastrin. Those tumors in which *G-17 binding was inhibited by secretin also possibly have a receptor for secretin that is distinct from the GR. (no Refs)

15. BINDING, INTERNALIZATION, AND DEGRADATION OF 125I-INSULIN BY CULTURED RAT PITUITARY TUMOR CELLS (MEETING ABSTRACT).

Corin RE, Bancroft FC, Donner DB
Memorial Sloan-Kettering Cancer Center, New York, NY
Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

A clonal line of rat pituitary tumor cells (GH3) 125 I-expressed specific, high affinity binding sites for 125I insulin. Native insulin (5nM) reduced receptor binding of 125I-insulin (54pm) by 50%. Crude plasma membranes prepared from GH3 cells contained trypsin-sensitive insulin binding sites. This suggested that at least part of the receptor population was localized to the surface of GH3 cells. GH3 cells degraded 125I-insulin in a time- and temperature-dependent manner. The inability of conditioned medium to degrade insulin and the observation of a lag time (5-10 min) before degradation by cells was detected indicated a cytoplasmic site of degradation. Chloroquine (1mM) and NH4Cl (10mM) inhibited degradation, suggesting a lysosomal locus of degradation. Excess native insulin (10--5M) completely inhibited the degradation of 125I-insulin (0.5-1.0nM), whereas growth hormone and ACTH (10--5M) had no effect upon degradation. The latter observation demonstrates a specificity for insulin degradation by GH3 cells. Exposure of 125I-insulin to GH3 cells followed by gel filtration of bound and free 125I-insulin showed that specifically bound hormone was degraded and the 125I-insulin fragments produced were transiently retained by the cells. Degradation of specifically bound 125I-insulin was inhibited by the flavin analog quinacrine (50-250uM), implicating a flavin-dependent process in the translocation of insulin from the cell surface to lysosomes. (no Refs)

TRANSPORT STUDIES

16. TRANSPORT OF D-GLUCOSE BY MEMBRANE VESICLES FROM NORMAL AND AVIAN SARCOMA VIRUS-TRANSFORMED CHICKEN EMBRYO FIBROBLASTS.

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Proc Natl Acad Sci USA; 78(9,part2):5358-5361 1981

Transport of D-glucose was examined in membrane vesicles from normal and avian sarcoma virus (ASV)-transformed chicken embryo fibroblasts. The initial rates of D-glucose transport were found to be 3-to 5-fold higher for vesicles from glucose-starved normal cells and ASV-transformed cells when compared with transport rates for vesicles from normal cells and serum-starved normal cells. Cytochalasin B, phloretin, and diethylstilbestrol inhibited the initial rate of transport in all types of vesicles, and 2-deoxyglucose, 3-O-methylglucose, and galactose were competitive inhibitors. At D-glucose concentrations between 0.25 and 5 mM, vesicles from normal and ASV-transformed cells displayed saturation kinetics with a Km value of 5 mM for both types of vesicles, with transformed cell vesicles showing a 3-fold increase in Vmax compared with normal cell vesicles. At D-glucose concentrations between 5 and 25 mM the initial rate of D-glucose transport was proportional to D-glucose concentration. The vesicles also showed an inhibitor-sensitive efflux at rates similar to those observed for influx. (Author abstract) (22 Refs)

17. RELATIVE FREQUENCY AND KINETIC PROPERTIES OF TRANSPORT-DEFECTIVE PHENOTYPES AMONG METHOTREXATE-RESISTANT L1210 CLONAL CELL LINES DERIVED IN VIVO.

Sirotnak FM, Moccio DM, Kelleher LE, Goutas LJ
Memorial Sloan-Kettering Cancer Center, New York, NY, 10021
Cancer Res; 41(11,part1):4447-4452 1981

Information was sought on the relative extent to which transport-defective, methotrexate-resistant phenotypes emerge among the total subpopulation of resistant phenotypes during therapeutic challenge of leukemic cells in vivo. A number of monoclonal methotrexate-resistant sublines of the L1210 leukemia were derived during methotrexate therapy of leukemic mice and biochemically characterized. Of the total number of 14 sublines derived, 5 exhibited altered [3H]methotrexate transport alone, 5 exhibited increased dihydrofolate reductase content alone (2-to 18-fold), and 4 showed alterations in both of these properties. Methotrexate binding and substrate turnover rate for dihydrofolate reductase appeared to be unchanged in any of the resistant sublines. The relative resistance of each subline was accounted for by the biochemical alterations observed. Among the transport-defective sublines, one subcategory showed a three- to four-fold reduction in apparent influx Vmax for [3H]methotrexate, a second category showed both a five-fold reduction in influx Vmax and a three-fold increase in the apparent influx Km, and one subline showed only a two-fold increase in Km. Otherwise, Michaelis-Menten saturation kinetics for influx was observed in each case and in the case of the parental line and the other resistant sublines. None of the resistant sublines exhibited altered efflux of [3H]methotrexate. Steady-state levels measured for intracellular exchangeable (osmotically active) fractions of drug accurately reflected the values for specific kinetic parameters determined for each sensitive and resistant cell line. These studies show that transport-defective phenotypes represent a major category of methotrexate-resistant cell types which emerge initially from leukemic cell populations under therapy in mice. Based on considerations discussed here, it is reasonable to assume that a similar relative occurrence of this phenotype would result during methotrexate therapy of leukemia patients. (Author abstract) (40 Refs)

18. CHARACTERIZATION OF THE METHOTREXATE TRANSPORT DEFECT IN A RESISTANT L1210 LYMPHOMA CELL LINE.

McCormick JI, Susten SS, Freisheim JH
Dept. Biological Chemistry, Univ. Cincinnati Coll. Medicine, Cincinnati, OH, 45267
Arch Biochem Biophys; 212(1):311-318 1981

Methotrexate (MTX) accumulation was examined in a MTX-resistant L1210 lymphoma c ell line (L1210/R81) in a buffer system lacking components inhibitory to MTX uptake and compared with MTX uptake in the parent, sensitive cell line (L1210/S), measured under the same conditions. L1210/R81

cells were MTX-resistant due to a 35-fold increase in dihydrofolate reductase levels and impaired ability to accumulate MTX. MTX transport studies were carried out in Hepes and bicarbonate buffer systems (Buffers 1 and 2, respectively). The L1210/S achieved a steady state in Buffer 2 at an intracellular MTX level of nanomoles (nmol)/10(9) cells, when the extracellular MTX concentration was 1 uM. A 4.6-fold increase in the level of 3H-MTX present L1210/S cells was observed when transport was measured in Buffer 1. L1210/R81 cells showed little or no drug uptake in Buffer 2 at 37 C. In Buffer 1, L1210/R81 cells accumulated 0.75 nmol 3H-MTX/10(9) cells during a 30 min incubation with an extracellular MTX concentration of 1 uM; steady state was achieved only when the extracellular MTX concentration was raised to 50 uM. In Buffer 1, p-chloromercuriphenylsulfonic acid initially stimulated, then inhibited 3H-MTX uptake in L1210/R81 cells. Sodium azide (100 uM) slightly inhibited 3H-MTX uptake in L1210/R81 in Buffer 1. In Buffer 1, both aminopterin inhibited 3H-MTX uptake in both L1210/R81 and L1210/S cells, while 5-methyltetrahydrofolate inhibited of 3H-MTX uptake in only L1210/S cells. Results indicated that L1210/R81 cells may contain functional receptors for MTX on the exterior surface of the plasma membrane that are expressed only in the absence of inhibitory ions in Buffer 2. However, unlike L1210/S cells, L1210/R81 cells may lack the functional components necessary for translocation of MTX across the plasma membrane into the intracellular compartment. (24 Refs)

19. RECIPROCAL REGULATION OF NA+-DEPENDENT HEXOSE AND NA+-DEPENDENT AMINO ACID UPTAKE IN LLC-PK1 CELLS: EFFECT OF 12-O-TETRADECANOYL-PHORBOL-13-ACETATE (TPA) (MEETING ABSTRACT).

Amsler K, Cook JS
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Thirteenth Miami Winter Symposium: Cellular Responses to Molecular Modulators, January 12-16, 1981, Miami, Florida. University of Miami 112 pp., 1981.

LLC-PK1 cells, an epithelial line derived from pig kidney, acquire the capacity for Na+-dependent concentrative alpha-methyl-D-glucopyranoside (alpha-meG, hexose) uptake as they reach the plateau phase of growth. Conversely, the cells during log phase growth have a high capacity for Na+-dependent concentrative uptake of alpha-aminoisobutyric acid (AIB, amino acid analog), which diminishes as the cells enter plateau phase. Growth of LLC-PK1 cells in the presence of 1 mM theophylline, 1 mM 1-methyl-3-isobutyl xanthine, or 2 mM hexamethylene bisacetamide (HMBA) accelerates both the expression of concentrative hexose uptake and the decrease in concentrative AIB uptake. The tumor promoter TPA added alone or with any of the above compounds retards both transport changes. Thus, 1) control cells after 6 days growth in culture are gaining hexose transport capacity and losing AIB-concentrating capacity. 2) Cells grown in HMBA-medium for 6 days have a high level of concentrative alpha-meG uptake and a concomitantly diminished AIB uptake when compared to control cultures. When TPA is added there is a rapid, cycloheximide-sensitive increase in the AIB accumulation capacity after addition, max at 6 hr, followed by a much slower decrease in concentrative alpha-meG uptake. 3) Cells grown 6 days in HMBA + TPA have no measurable hexose concentrating capacity but a high level of concentrative AIB uptake. Transfer of such cells to HMBA medium without TPA leads to a slow increase in alpha-meG uptake and a slow loss of AIB concentrating capacity; neither change is observed until at least 24 hr after TPA removal. (no Refs)

20. EFFECT OF THE TUMOR PROMOTER 12-O-TETRADECANOYLPHORBOL-13-ACETATE ON ALPHA-AMINOISOBUTYRATE UPTAKE IN FRIEND ERYTHROLEUKEMIC CELLS.

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Cancer Res; 41(11,part1):4512-4517 1981

In Friend erythroleukemic cells, the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) increases the rate of influx as well as the steady-state accumulation of the amino acid transport probe alpha-aminoisobutyric acid (AIB). This non-metabolizable analog is concentrated in these cells by utilizing the energy of the Na+ electrochemical gradient. The effect of TPA is to increase the ouabain-sensitive component of uptake of AIB (and of the K+ analog 86Rb+). Transport changes are not observed until 30 min after application of the promoter and reach a max in about 6 hr. The effects are blocked by cycloheximide. The flux results are consistent with a model in which the membrane potential is increased and, with it, the driving force on the coupled Na+ -AIB movement. The effect is possibly mediated by a change in electrogenicity of the Na:K pump. TPA raises the steady-state accumulation levels of AIB. By altering external K+ in the presence of valinomycin, the membrane potential may be adjusted and, with it, the accumulation of AIB. Over a wide range of membrane potentials, TPA-treated cells accumulate more AIB than do controls, suggesting a possible change in the internal Na+ activity. (Author abstract) (35 Refs)

21. CHANGES IN TRANSMEMBRANE POTENTIAL OF LEUKOCYTES OF CANCER PATIENTS AND MODULATION BY LEUKOTRIENES AND PROSTAGLANDINS (MEETING ABSTRACT).

Shenouda G, Thomson DM, Bach MK
No affiliation given
International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

In our laboratory, tumor antigen (TA)-induced WBC adherence inhibition (LAI) by a tube assay has been used to detect human antitumor immunity. To correlate the LAI phenomenon with physiological changes in the WBC (PBL), we undertook to measure TA-induced differences in transmembrane potential (TMP) of PBL, using tetraphenylphosphonium ion, a lipid-soluble cation, that equilibrates rapidly across biological membranes and reflects the magnitude and direction of a potential difference. PBL from cancer patients when incubated with the sensitizing TA manifested rapid changes in TMP over 15 min, including depolarization and hyperpolarization. PBL from control subjects exhibited no change in TMP to TA. Likewise, PBL from patients with advanced cancer did not show changes; however, if these PBL were briefly incubated with PGE2 or aminophylline, they then responded. Purified monocytes armed with cytophilic antitumor antibody, when incubated with the sensitizing TA, released a mediator that induced TMP changes of PBL. FPL 55712 inhibits the induction of TMP changes by the mediator, and 5,8,11,14-eicosatetraynoic acid inhibited the synthesis of the mediator by monocytes. Pure leukotriene C and leukotriene D induce transmembrane potential changes of WBC both mononuclear and PMNs that closely resembles the pattern induced by TA. The results of this study indicate that TA can induce changes in transmembrane potential of sensitized PBL. Monocytes and the released

leukotrienes play an important role in the TA-induced transmembrane potential changes. (no Refs)

MEMBRANE BIOGENESIS AND METABOLISM

22. MEMBRANE GLYCOPROTEINS INVOLVED IN CELL-SUBSTRATUM ADHESION.

Knudsen KA, Rao PE, Damsky CH, Buck CA
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Proc Natl Acad Sci USA; 78(10):6071-6075 1981

A combination of immunological and biochemical methods were used to identify surface membrane components involved in cell-substratum adhesion. Broad-spectrum antiserum, prepared against surface membranes from hamster cells, induced reversible rounding and detachment of hamster fibroblasts from a substratum in vitro. This phenomenon was inhibited by Nonidet P-40 extracts of hamster cells. Therefore, an antibody neutralization assay was developed to detect the presence of antigen during the fractionation of Nonidet P-40 extracts of cells. After two differential precipitation steps, anion exchange chromatography, and sequential lectin affinity chromatography, a fraction greatly enriched in ability to block antiserum-induced changes in cell adhesion and appearance was isolated. Analysis of this fraction by NaDodSO4/polyacrylamide gel electrophoresis revealed a highly restricted group of glycoproteins with Mr = approx 140,000. A lectin-purified glycoprotein fraction was used to raise a higher titer antiserum that was able to induce reversible rounding and detachment of cells from a substratum and, when immobilized on an antibody affinity column, was able to bind and release material capable of blocking antiserum-induced cell rounding. These methods have allowed us to focus attention on a restricted group of glycoproteins that are integral constituents of the surface membrane and which play some as yet undetermined role in the process of cell-substratum adhesion. (Author abstract) (31 Refs)

23. MEMBRANE DIFFERENTIATION IN HUMAN MYELOID CELLS: EXPRESSION OF UNIQUE PROFILES OF CELL SURFACE GLYCOPROTEINS IN MYELOID LEUKEMIC CELL LINES BLOCKED AT DIFFERENT STAGES OF DIFFERENTIATION AND MATURATION.

Fukuda M, Koeffler HP, Minowada J
Biochemical Oncology, Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
Proc Natl Acad Sci USA; 78(10):6299-6303 1981

Cell surface glycoprotein structures of human leukemic cell lines blocked at various stages of myeloid (granulocyte-monocyte) differentiation and maturation were characterized. Cell lines representing early stages of differentiation and maturation such as KG1a, KG1 and those representing later stages such as ML-1-3 and HL-60 were found to express discretely different sets of cell surface glycoproteins. The proteins (such as Gp130) present on mature granulocytes appear only in cell lines representing later stages of maturation. The quantity and structure of the carbohydrate chains susceptible to endo-beta-galactosidase ('lactosaminoglycan') are also significantly different among cell lines. A small quantity of essentially unbranched lactosaminoglycan is present on KG1a and KG1 cells, whereas a significant quantity of branched lactosaminoglycan is present on the more mature ML-1-3 and HL-60 cells. The process of myeloid maturation from promyeloblast to granulocyte is accompanied by the disappearance of the and 95-kilodalton glycoproteins, the appearance of Gp130, and a great increase in the quantity and branching structure of lactosaminoglycan. These results are discussed together with our previous findings on erythroid differentiation and maturation. (Author abstract) (36 Refs)

24. USE OF CELL DIFFERENTIATION EFFECTS TO SELECT A HUMAN B LYMPHOBLASTOID CELL LINE ENRICHED IN C3B RECEPTORS.

Barel M, Charriaut C, Frade R
(c/o Frade), Hopital Paul Brousse, I.C.I.G. INSERM U. 50 and Association Claude Bernard, 94800 Villejuif, France
J Immunol Methods; 46(2):187-195 1981

A study was undertaken to establish conditions of growth to increase C3b receptor synthesis on a human B lymphoblastoid cell line (Raji) by use of cell differentiation effectors. It appears that whereas two polar compounds HMBA (2 mM) and Me2SO (2%) have no or little effect, 5 BrdU (30 uM) and db cAMP (5 x 10(-4) M) are able to increase in 48 hr and 36 hr respectively the synthesis of C3b receptor o n Raji cell surface. These two compounds help to select a variant in which 100% of cells have C3b receptors with a high density of receptors per cell. The mechanism of BrdU action on the regulation of C3b receptor synthesis is discussed. (Author abstract) (23 Refs)

25. TWO HUMAN EXOCRINE PANCREATIC CANCERS ESTABLISHED IN ATHYMIC MICE: SECRETORY ENZYMES, MEMBRANE RECYCLING, AND SOME HORMONE RECEPTORS (MEETING ABSTRACT).

Estival A, Balas D, Clemente F, Ribet A
INSERM U 151 CHU Rangueil, 31400 Toulouse, France
Gastroenterology; 80(5,part2):1143 1981

Two human pancreatic adenocarcinoma (Kc P1, Kc P2) with different growth rates were established as xenographs in nude mice. The existence of normal secretory enzymes in the tumor secretory granules was analyzed using immunocytochemical techniques, with antibodies directed against normal human pancreatic juice. The plasma membrane turnover was studied using cationic ferritin. Endocytic vesicles are quickly labelled and reach the inner golgian zone in the 10th min. Numerous endocytic vesicles containing ferritin were directly fusioning with condensing vacuoles. From the 30th to the 120th min the tracer was also observed in condensing vacuoles and secretory granules. These findings suggest a short pathway and recycling of plasma membrane components probably related with a defect on the first stages of protein synthesis. Some hormone receptors were also analyzed in the two established tumors. Vasoactive intestinal peptide (10-7M) and carbachol (10-6M) respectively increase cyclic (c) AMP and cyclic guanosine monophosphte (cGMP) levels. Secretin stimulates adenylate cyclase activity, the maximal response is reached at high hormone concentrations 10-5M -10-6M. Dopamine increases the cAMP level with a maximal response for 10-5M. Caerulein at concentration as low as 10-11M stimulates guanylate cyclase activity. In conclusion: 1) Normal secretory enzymes were not found. 2) Abnormal membrane recycling is demonstrated. 3) The two human cancers possess carbachol, dopamine, caerulein and secretin receptors; the responses evoked by the two last hormones resemble that of acinar cells more than that of duct cells. (no Refs)

26. EFFECTS OF DEXAMETHASONE ON LIPID METABOLISM IN HUMAN CHRONIC LYMPHATIC LEUKEMIA CELLS (MEETING ABSTRACT).

Adams SE, Melnykovych G
University of Kansas Medical Center, Kansas City, KS, 66103
In Vitro; 17(3):232 1981

Dexamethasone (dex) causes a 25% inhibition of growth of a human chronic lymphatic leukemia (CLL) cell line following two days of exposure. Although the basis of this growth inhibition is not known, a number of changes in lipid metabolism have been observed in cells exposed to dex. A 35% inhibition of 3H-acetate incorporation into the non-saponifiable lipid fraction of CLL cells was observed after one hr of exposure to dex. Acetate incorporation into the saponifiable lipids was decreased by 20% after two hr of exposure to dex followed by a 20% inhibition of 3H-thymidine incorporation into trichloracetic acid precipitable material after four hr of exposure to steroid. This later resulted in a 40% decrease in total cellular phospholipid content although cellular cholesterol levels remained unaffected. In spite of the decreased phospholipid content, a 24 hr exposure of CLL cells to dex caused a 2-fold stimulation of cytidine diphosphate-choline transferase activity which corresponded with a 25% increase in choline incorporation into phosphatidyl choline. However choline incorporation into sphingomyelin was inhibited by 50%. The resultant alteration in the sphingomyelin/phosphatidyl choline ratio of CLL membranes may be an important factor in the growth inhibition caused by dex. (no Refs)

CELL ADHESION

27. GALACTOSYL SPECIFIC RECEPTOR ON LIVER CELLS: BINDING SITE FOR TUMOR CELLS.

Schlepper-Schafer J, Friedrich E, Kolb H
Institut fur Biophysik und Elektronenmikroskopie der Universitat, Moorenstr. 5, D-4000 Dusseldorf 1, W. Germany
Eur J Cell Biol; 25(1):95-102 1981

In this paper the recognition of various rat tumor cells by rat liver cells is demonstrated in vitro. A liver cell receptor involved in the binding process has been identified. The ultrastructure of the cell contacts was examined by transmission electron microscopy. Hepatocytes and Kupffer cells were isolated from rat liver by collagenase treatment and cell adhesion tests were performed with four different tumor cell types. Hepatocytes were found to bind Walker sarcoma cells, lymphoma cells and Yoshida hepatoma cells but not leukemia 5222 cells. Kupffer cells bound all tumor cell types. Normal blood cells were not bound under the same conditions. Recognition of tumor cells by hepatocytes was mediated by a galactose specific lectin on the liver cell surface as shown by hapten inhibition experiments with specific saccharides. Although Kupffer cells express a similar lectin-like receptor adhesion of tumor cells, they could not or could only slightly be inhibited by galactose or related saccharides. It is concluded that the spontaneous adhesion of tumor cells to liver cells in vitro is a specific recognition event which in part is mediated by lectin-carbohydrate interactions. (Author abstract) (33 Refs)

28. SPECIFIC ROSETTE FORMATION BETWEEN FIBROBLASTS AND ERYTHROCYTES.

Rosen SD, Singer MS, Glabe CG, Grabel LB
Dept. Anatomy, Biochemistry and Biophysics, Univ. California Medical Center, San Francisco, CA, 94143
J Cell Sci; 51:279-294 1981

Fibroblast-like cells (BHK-21, Swiss 3T3, SV40 3T3, L929) will form rosettes by binding to RBC of specific types. The specificity of binding varies with the cell line. Monolayer cultures of spread baby hamster kidney (BHK) cells also bind RBC with the same specificity exhibited by suspended cells. Our studies have concentrated on rosette formation between trypsinized-ox (TOx) RBC and BHK cells. Extensive protease treatment of the BHK cells reduces their ability to bind TOx RBC in a dose- and time-dependent manner, suggesting the involvement of protein in the binding site. Simple saccharides, and glycopeptides isolated form ox RBC ghosts, do not inhibit rosetting. However, neutral glycosphingolipids from ox RBC ghosts inhibit BHK-TOx rosetting, whereas neutral glycosphingolipids from non-rosetting rabbit RBC have no effect on rosetting. Furthermore, incorporation of ox glycolipids into guinea pig RBC causes these non-rosetting RBC to adhere to BHK cells. These experiments suggest that specific glycolipids of the ox RBC act as receptors for binding sites on the cell surface of BHK cells. These cell-specific binding sites may play a role in cell-surface events involving carbohydrate recognition. (Author abstract) (42 Refs)

29. TUMOR CELL INTERACTIONS WITH VASCULAR ENDOTHELIAL CELLS AND THEIR EXTRACELLULAR MATRIX (MEETING ABSTRACT).

Nicolson GL, Kramer RH
Department of Oral Medicine, University of San Francisco, San Francisco, CA. 94143
Chesapeake Conference on Hemostasis and Malignancy-Interaction of Platelets and Tumor Cells, June 26-30, 1981, St Mary's Maryland American Red Cross; 1981

In order for distant blood-borne metastasis to form, circulating malignant cells must bind to the vascular endothelium and invade the underlying basement membrane. A model for this process has been developed using cultured vascular endothelial cell monolayers which synthesize a basolateral extracellular matrix or basal lamina. Metastatic tumor cells, as well as some normal cells such as WBC bind to vascular endothelial monolayers and stimulate endothelial cell retraction, exposure of the underlying extracellular matrix, migration of the malignant cells to the matrix surface and finally the destruction of the matrix. The movement of metastatic tumor cells to endothelial extracellular matrix appears to be due to an adhesive difference between the endothelial cell and the matrix surface. Human and murine melanoma cells adhere much more rapidly to the extracellular matrix, and this can be mimicked by the attachment of tumor cells to polyvinyl-immobilized fibronectin. Anti-fibronectin inhibits slightly binding of melanoma cells to matrix, but completely blocks binding to immobilized-fibronectin suggesting that this molecule may mediate only in part the enhanced adhesion of melanoma cells to matrix. Tunicamycin inhibits glycosylation of melanoma cell glycoproteins and blocks tumor cell-endothelial cell attachment in vitro and blocks experimental metastasis in in vivo. Metastatic cells release degradative enzymes which are capable of so lubilizing the major components of the endothelial extracellular matrix: glycoproteins such as fibronectin and lamanin, collagens and sulfated proteoglycans. Tumor cells that lack these enzymes

may not be effective in escaping the vascular compartment. (3 Refs)

30. INTERACTIONS OF TUMOR CELLS WITH WHOLE HUMAN BASEMENT MEMBRANE IN THE PRESENCE OR ABSENCE OF ENDOTHELIUM (MEETING ABSTRACT).

Foltz CM, Segal GP, Russo RG, Terranova VP, Liotta LA

Laboratory of Pathophysiology, National Cancer Institute, Bethesda, MD, 20205

Chesapeake Conference on Hemostasis and Malignancy-Interaction of Platelets and Tumor Cells, June 26-30, 1981, St Mary's Maryland American Red Cross; 1981

A new method has been developed to study in vitro the interaction of tumor cells and endothelial cells with whole human basement membrane (BM). Human amnion BM is prepared as a continuous native surface by removal of the epithelial cells from the amnion membrane. The attachment rates of human tumor cells and human endothelium to the BM is studied in the presence or absence of the attachment factors, fibronectin and laminin, or after selective enzymatic treatment of the BM. Radioactively labeled whole BM is also prepared by organ culture of aminon incubated with 14C-amino acids. Tumor cells are found to utilize laminin for BM attachment. Tumor cell attachment to BM is followed by BM degradation. The biochemical studies are supplemented by immunohistology. (no Refs)

31. INHIBITION OF TUMOR CELL ADHERENCE BY PGE1 AND PGI2 (MEETING ABSTRACT).

Fantone J, Kunkel S, Varani J

Department of Pathology, University of Michigan School of Medicine, Ann Arbor, MI, 48109

International Conference on Prostaglandins and Cancer, August 30-September 2, 1981, Washington, DC. Lombardi Cancer Center 75 pp., 1981.

Prostaglandins (PGs) and thromoboxanes are known to be potent mediators of both neutrophil and platelet adhesiveness. Several reports suggest that the potent anti-aggregatory PGs, PGI2 and PGD2, decreased tumor cell metastasis by inhibiting platelet adherence to tumor cells thereby blocking the adherence of the tumor cell-platelet aggregates to endothelium. Recent in vitro studies in our laboratory have shown that Walker Carcinosarcoma Cells (WCC) show increased adherence to plastic plates and bovine corneal endothelium when stimulated with chemotactic agents, N-formyl-methionyl leucyl-phenylalanine and trypsinized fragments of C5, as well as phorbol myristate acetate (PMA), a potent membrane stimulator. When WCC are pre-treated with either PGE1 or PGI2 in the presence of theophylline there is 56.8 +/-4.8% and 50.4 +/-4.2% inhibition of PMA stimulated adherence to plastic plates. Similar effects are observed when WCC are pre-treated with either histamine or dibutyryl cyclic AMP (cAMP). These studies show that the anti-aggre gatory PGs, PGE1 and PGI2 can inhibit tumor cell adhesiveness in vitro and suggest that the inhibition of metastasis may be due to the direct effect of these agents on the tumor cell. These effects may be mediated through alteration of intracellular cAMP levels. (no Refs)

32. INTERACTION OF PLATELETS AND TUMOR CELLS (MEETING ABSTRACT).

Steiner M

Division of Hematologic Research, The Memorial Hospital, Pawtucket, RI, 02860

Chesapeake Conference on Hemostasis and Malignancy-Interaction of Platelets and Tumor Cells, June 26-30, 1981, St Mary's Maryland American Red Cross; 1981

In a series of studies, certain tumor cells were shown capable of aggregating platelets, while platelets undergoing aggregation were found to support tumor cell proliferation. Two lines of mouse tumor cells (renal adenocarcinoma, RAG and neuroblastoma, Neuro-2a) capable of aggregating mouse or rabbit platelets were investigated. Specific divalent cation requirements were identified for the tumor cell-induced platelet aggregation. This activity was associated with the tumor cell membrane and was also characterized by very specific structural constraints. Aggregation was accompanied by a secretory release reaction, products of which were found to have growth-promoting activity for tumor cells. Lysates of human platelets provided an excellent source for this factor, which was able to support tumor cell proliferation in the absence of plasma or serum. The activity was non-dialysable, heat-stable at 56 degrees, partially trypsin-labile and sensitive to periodate oxidation. One or more peptides with strong growth-promoting activity coud be isolated by isoelectric focusing. These peptides are not identical with the platelet-derived growth factor with activity for non-malignant cells. (no Refs)

33. PLATELET AGGREGATING MATERIAL (PAM) OF TWO VIRALLY-TRANSFORMED TUMORS: SV3T3 MOUSE FIBROBLAST AND PW20 RAT RENAL SARCOMA - ROLE OF CELL SURFACE SIALYLATION (MEETING ABSTRACT).

Karpatkin S, Pearlstein E, Salk PL, Yogeeswaran G

New York University Medical Center, School of Medicine, New York, NY, 10016

Chesapeake Conference on Hemostasis and Malignancy-Interaction of Platelets and Tumor Cells, June 26-30, 1981, St Mary's Maryland American Red Cross; 1981

We have extracted a sedimentable stalo-lipo-protein, platelet aggregating material (PAM) from the cell surface of SV40 transformed Balb C3T3 fibroblasts which aggregates heparinized platelet-rich plasma at 2.5 ug/ml via the release reaction, following a 1 min lag period. Gel-filtered platelets (GFP) do not aggregate with PAM. However, PAM aggregation can be restored by addition of 5% plasma but not by fibrinogen. The lag period can be abolished by preincubation of PAM with a plasma factor for 10 min at 37 C. This plasma factor is labile to heating at 56 C for 30 min. When PAM is coupled to sepharose beads treated with cyanogen bromide, platelets from PRP adhere to these beads suggesting that PAM binds to platelets. GFP platelets do not adhere to the beads unless plasma is added. Platelets from PRP or GFP plus plasma do not adhere to beads coupled to albumin or fibronectin. The pathophysiologic significance of PAM has been examined in ten variant cell lines derived from a spontaneously metastatic renal cell sarcoma of rats, initially induced with polyoma virus (PW20 Wistar-Furth parental lines). These lines were selected in vitro and in vivo from a single line and differed in their capacity to form distant tumors in various organs after sc injection. These cells were examined for cell surface sialylation. PAM and PAM sialic acid content, since cell surface sialic acid is increased in a variety of tumor tissues and PAM is inhibited by neuraminidase. We conclude that platelets may play a role in hematogenous metastasis via the ability of tumor cells to aggregate pla-

telets by cell surface constituents containing sialic acid. The platelet-tumor cell interaction requires activation of the alternate complement pathway and a heat stable plasma factor. (no Refs)

34. SERUM SPREADING FACTOR: EFFECTS ON RF1 RAT OVARY CELLS AND 1003 MOUSE EMBRYONAL CARCINOMA CELLS IN SERUM-FREE MEDIA (MEETING ABSTRACT).

Barnes DW, Darmon M, Orly J
Department of Biology Science, University of Pittsburgh, Pittsburgh, PA, 15260
Growth of Cells in Hormonally Defined Media, September 1-4, 1981, Cold Spring Harbor, New York Cold Spring Harbor Laboratory 101 pp., 1981.

Serum spreading factor (SSF), a glycoprotein partially purified from human serum, influences the growth and morphology of a number of fibroblastic and epithelial cell types in serum-free media (SFM). The major portion of the protein in the SSF preparations is estimated to be a mol wt between 60,000 and 90,000 by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) of reduced samples. SSF is similar to fibronectin or cold-insoluble globulin (CIg) in that it shows an affinity for the plastic cell culture substratum, but appears to be distinct from CIg by several criteria. Previously CIg at 0.5 to 5 ug/ml was shown to mediate cytokinesis and growth of RF1 rat ovary cells in SFM supplemented with insulin (I), transferrin (TF) and hydrocortisone. Similar results are obtained if CIg is replaced with SSF preparations in the same concentration range. The 1003 mouse embryonal carcinoma cell line is a multipotential line which differentiates predominately into neurons in SFM. Addition of 5 ug/ml SSF to 1003 cells in SFM supplemented with I, TF and CIg results in increased saturation density, increased spreading of islets of cells on the dish and delayed differentiation compared to cells in the absence of SSF. In the absence of CIg, 1003 cells do not attach to the dish in the presence of SSF. (2 Refs)

35. A LIGHT AND ELECTRON MICROSCOPIC STUDY ON REAGGREGATION OF RAT ASCITES HEPATOMA CELLS MAINTAINED IN VITRO.

Koga Y
First Dept. Pathology, Kumamoto Univ. Medical Sch., Kumamoto, Japan
Kumamoto Med J; 34(2):79-90 1981

The ability of AH136B rat ascites hepatoma cells (a regenerator of cell surface-associated adhesive factor) to adhere to each other and then develop the binding structures closely resembling those observed on island-forming cells is described. It was suggested that dissociated AH136B cells themselves produce the adhesive factor, and were led to reaggregation and development of binding structures. (34 Refs)

OTHER RELATED STUDIES

36. INTERFERON INCREASES THE ABUNDANCE OF SUBMEMBRANOUS MICROFILAMENTS IN HELA-S3 CELLS IN SUSPENSION CULTURE.

Wang E, Pfeffer LM, Tamm I
Rockefeller Univ., New York, NY, 10021
Proc Natl Acad Sci USA; 78(10):6281-6285 1981

Human beta(fibroblast) interferon inhibits the proliferation of human HeLa-S3 carcinoma cells in suspension culture. Accompanying this effect, the lateral mobility of cell surface receptors for concanavalin A is decreased and the rigidity of the plasma membrane lipid bilayer is increased. The present findings show a marked increase in the number of polymerized actin-containing microfilaments 3 days after treatment of HeLa-S3 cells with beta-interferon (640 units/ml). The cortical region of the treated enlarged cells contains a thick and dense meshwork of 40-70 A microfilaments. The actin nature of the filaments was verified by their ability to bind heavy meromyosin. These results support the concept that beta-interferon induces a coordinated response in the plasma membrane and the underlying microfilaments in both tumor and normal cells. (Author abstract) (35 Refs)

37. TUMOR PROMOTERS CAUSE A RAPID AND REVERSIBLE INHIBITION OF THE FORMATION AND MAINTENANCE OF ELECTRICAL CELL COUPLING IN CULTURE.

Enomoto T, Sasaki Y, Shiba Y, Kanno Y, Yamasaki H
Dept. Physiology, Hiroshima Univ. Sch. Dentistry, Hiroshima 734, Japan
Proc Natl Acad Sci USA; 78(9,part2):5628-5632 1981

The effect of tumor promoters on electrical coupling between human FL cells was investigated with a microelectrode technique. When a low concentration (100 ng/ml) of 12-O-tetradecanoylphorbol 13-acetate (TPA) was added to culture medium, only 6% of the cells showed electrical coupling after 5 hr, whereas in control medium more than 90% of the cells were coupled. In the presence of TPA, cell coupling remained suppressed for at least another 19 hr. When TPA was washed out from the culture medium, the cells commenced electrical coupling: 90% of the cells were coupled within 4 hr of the removal of TPA, a rate very similar to that of nontreated control cells. Therefore, TPA-mediated inhibition of cell coupling is reversible. When TPA was added to a culture in which more than 90% of the cells had already established electrical coupling, the percentage of coupled cells decreased to 6% within 8 hr, indicating that TPA can also diminish already established cell coupling. Inhibition of cell coupling also was achieved with other mouse skin tumor promoters - phorbol 12,13-didecanoate, ingenol dibenzoate, and mezerein - whereas nonpromoting derivatives - phorbol and 4alpha-phorbol 12,13-didecanoate - showed no effect. None of these compounds changed the membrane potential, membrane resistance, or growth rate of FL cells. Thus, it appears that TPA and structurally-related tumor promoters specifically disturb the formation or function, or both, of cell-cell junctions, without significantly affecting the general properties of the surface membrane or the growth of FL cells. (Author abstract) (43 Refs)

38. PLASMA MEMBRANE VESICLES AS MEDIATORS OF INTERACTIONS BETWEEN TUMOR CELLS AND COMPONENTS OF THE HEMOSTATIC AND IMMUNE SYSTEMS (MEETING ABSTRACT).

Gasic G, Gasic T

Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, 19140

Chesapeake Conference on Hemostasis and Malignancy-Interaction of Platelets and Tumor Cells, June 26-30, 1981, St Mary's Maryland American Red Cross; 1981

Cells from a variety of mouse and rat tumors can shed plasma membrane vesicles. In vitro experiments indicate that these vesicles can interact with blood platelets and macrophages. Here we report species differences affecting platelet-vesicle interactions and the effects of new compounds on these interactions. Also we raise the hypothesis that vesicle surface material active in platelet aggregation (PA) might also influence immunogeneticity of tumors via vesicle-macrophage interactions. In the rat system, addition of vesicles to platelet rich plasma regularly induce PA. This phenomenon is preceded by binding of vesicles to platelets. Decomplementation blocks both binding and PA. Coumadin reduction of clotting factors and thrombin inhibitors inhibit PA but not binding. In mice, which are widely used as experimental model in thrombogenesis, metastasis, and tumor immunity, platelet responses to vesicles are less predictable and depend on genetic factors affecting complement, clottability, platelet receptors, or a combination of these factors. PA in mice can be fully inhibited in vitro and in vivo by synthetic anti-thrombin compounds and by invertebrate salivary gland extracts. In a syngeneic system, vesicles from a highly immunogenic tumor interacted with macrophages which upon injection into mice enhanced their resistance to sbc tumor grafts. This observation led us to reexamine unpublished data which show a good correlation between tumor immunogenicity and PA activity. It remains to be established whether vesicles released by circulating or invading t umor cells can also successfully interact in vivo with macrophages and which role platelets may play in this interaction. (1 Ref)

39. NONSPECIFIC INHIBITORY ACTIVITY OF SOLUBLE HUMAN COLON CARCINOMA EXTRACTS: TENTATIVE MECHANISM OF ACTION.

Pommier GJ, Remacle-Bonnet MM, Rance RJ, Depieds RC
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JNCI; 67(4):791-802 1981

Soluble human colon carcinoma extract(s) (SCE) were potent nonspecific inhibitors of lymphoproliferative responses to mitogens. Inhibition was concomitant with induction in about 35% of cells of morphologic alterations for most of them comparable with the ones observed in mitogen-induced blast cells. Nonetheless, these blastlike cells did not proliferate. SCE did not interfere with mitogen binding to cell receptors. Moreover, SCE was unable to induce or activate suppressor cells, and its primary target cell was the unresponsive lymphoid cell itself. The inhibitory effect of SCE was early and irreversible. The differential activity of SCE can be correlated with an early [3H]uridine uptake, which was inhibited 6 hr later, as seen for the other biochemical parameters of cell activation. Also, SCE altered membrane-bound ATPase activities. Na,K-ATPase was strongly inhibited, whereas Ca++-dependent and Mg++-dependent ATPases were stimulated. These observations were discussed as an SCE-lymphocyte ***plasma membrane interaction*** translated into differential signals to the intracellular metabolic pathways. (Author abstract) (62 Refs)

40. THE ROLE OF METALS IN CARCINOGENESIS: BIOCHEMISTRY AND METABOLISM.

Jennette KW
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Environ Health Perspect; 40:233-252 1981

A review is presented of the biochemistry and metabolism of inorganic species involved in carcinogenesis. Such species may have a role in transport across the cell membrane, enzymatic and chemical transformation of redox active inorganic species within the cell, coordination to cellular small molecules and macromolecules, and inhibition, activation or change of specificity of cellular enzymes by inorganic species. Oxide complexes are discussed with respect to enzyme interaction, metabolism, and mutagenicity. The role of divalent ions is examined. Oxyanions of V, Cr, Mo, As, and Se cross cell membranes; once inside the cell they may act as phosphate and/or sulfate analogs, inhibiting enzymes which catalyze phosphoryl or sulfuryl transfer reactions. They may serve as alternate enzyme substrates, but they form ester products which are hydrolytically unstable and decompose readily in aqueous soln. The divalent ions of Be, Mn, Co, Ni, Cd, Hg, and Pb are stable forms which may mimic essential divalent ions such as Mg, Ca, Fe, Cu, or Zn. These ions may complex small molecules, enzymes, and nucleic acids so as to alter the normal activity of these species. Free radicals produced in the presence of these metal ions may damage critical cellular molecules. (216 Refs)

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STRUCTURAL AND FUNCTIONAL ASPECTS OF CELL MEMBRANES

SERIES CB06 No. 82/04

APRIL, 1982

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. ASSOCIATION OF A 68,000-DALTON PROTEIN WITH ADRENOCORTICOTROPIN-SENSITIVE ADENYLATE CYCLASE ACTIVITY IN Y1 ADRENOCORTICAL TUMOR CELLS.

Watt VM, Schimmer BP
Banting and Best Dept. Medical Res., Univ. Toronto, Toronto, Ontario M5G 1L6, Canada
J Biol Chem; 256(21):11365-11371 1981

This report explores the biochemical basis for clonal variation in adrenocorticotropin (ACTH)-sensitive adenylate cyclase activity in the Y1 mouse adrenocortical tumor cell line. We demonstrate that the level of a specific protein, designated p68, is significantly correlated with the ability of adrenocorticotropin to stimulate adenylate cyclase activity among Y1 sub-

HE 20.3173/2:

clones ($p = 0.004$; $r = 0.65$). p 68 was characterized by its mol wt in sodium dodecyl sulfate polyacrylamide gels (Mr = 68,000) and by its isoelectric point as determined by two-dimensional gel electrophoresis (pI = 7.2). On two-dimensional gels, the protein migrated as a major spot with satellite spots 0.1 pH unit on either side. Homogenates and plasma membrane fractions from clones highly responsive to ACTH had large amounts of p68. In homogenates from highly responsive clones, p68 represented 10 to 12% of the total protein. Homogenates and plasma membrane fractions from clones insensitive to ACTH were deficient in p68. In homogenates from the insensitive clones Y6 and OS3, p68 represented less than or equal to 0.8% of the total protein. A somatic cell hybrid, formed by fusion of these two ACTH-insensitive clones recovered ACTH-sensitive adenylate cyclase activity and concomitantly expressed appreciable levels of p68. It is suggested that p68 may regulate the transfer of information from the occupied ACTH receptor to the catalytic subunit of adenylate cyclase. (Author abstract) (28 Refs)

2. TYROSYLPROTEIN KINASE AND PHOSPHATASE ACTIVITIES IN MEMBRANE VESICLES FROM NORMAL AND ROUS SARCOMA VIRUS-TRANSFORMED RAT CELLS.

Gallis B, Bornstein P, Brautigan DL
Dept. Biochemistry, Univ. Washington, Seattle, WA, 98195
Proc Natl Acad Sci USA; 78(11,part2):6689-6693 1981

Membrane vesicles, isolated from normal and Rous sarcoma virus-transformed rat cells, have an associated cyclic-AMP independent kinase that phosphorylates a Mr 37,000 protein in vesicles from normal cells and proteins of Mr 37,000, 50,000, and 67,000 in vesicles from transformed cells. Proteins in vesicles from normal and transformed cells contain 9% and 77%, respectively, of their labeled phospho amino acids as phosphotyrosine. Thus, isolation of vesicles and subsequent incubation with [gamma-32P]ATP enriches the proportion of labeled phosphotyrosine in proteins (relative to other phospho amino acids) by two orders of magnitude over that found in intact cells. The in vitro phosphorylation of each of these proteins is enhanced in the presence of 10 uM Zn++, a phosphotyroslyprotein phosphatase inhibitor. From these studies it appears that membrane vesicles may be a valuable system for examination of transformation-specific phosphorylation of proteins. (Author abstract) (25 Refs)

3. ROLE OF TUMOR CELL MEMBRANE-BOUND SERINE PROTEASES IN TUMOR-INDUCED TARGET CYTOLYSIS.

DiStefano JF, Beck G, Lane B, Zucker S
Dept. Res., Veterans Admin. Medical Center, Northport, NY, 11768
Cancer Res; 42(1):207-218 1982

The tumor-induced marrow and RBC cytolysis assays have been used to explore the mechanism of cancer cell destruction of normal cells. Previously, we suggested that tumor-induced cytolysis was caused by tumor cell membrane-bound serine proteases. In this study, we have shown that concentration s of the broad-spectrum serine protease inhibitor diisopropylfluorophosphate that did not inhibit tumor cell DNA and protein synthesis completely abrogated tumor-induced RBC cytolysis. In addition, tumor cell membranes isolated by differential and sucrose density gradient centrifugation and characterized by electron microscopy and enzyme marker analysis were cytolytic for rat 59-Fe-labeled RBC. The specific activity expressed as release index (%) per ug of protein was 1.620 for the tumor cell membrane preparations as compared to 0.002 for intact Walker 256 tumor cells. Tumor cell membranes solubilized in Triton X-100 had activity in the p-toluenesulfonyl-L-arginine methyl ester assay for trypsin-like enzymes and the N-benzoyl-L-tyrosine ethyl ester assay for chymotrypsin-like enzymes. The enzyme activities demonstrated in these assays could be inhibited by N-alpha-p-tosyl-L-lysine chloromethyl ketone HCl and L-1-tosylamide-alpha-phenylethyl chloromethyl ketone, respectively. Using [3H]diisopropylfluorophosphate affinity labeling of the tumor cell membrane proteins followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, we have identified membrane-bound serine protease(s) that appear to be responsible for tumor-induced marrow and RBC cytolysis. (Author abstract) (53 Refs)

4. CONTINUED EXPRESSION OF VINCA ALKALOID RESISTANCE BY CCRF-CEM CELLS AFTER TREATMENT WITH TUNICAMYCIN OR PRONASE.

Beck WT, Cirtain MC
Div. Biochemical and Clinical Pharmacology, St. Jude Children's Res. Hosp., 332 North Lauderdale, Post Office Box 318, Memphis, TN, 38101
Cancer Res; 42(1):184-189 1982

We have shown that a glycoprotein(s) with a mol wt of approx 180,000 exists on the surface of cultured lymphoblasts selected for resistance to vinblastine (VLB) (CEM/VLB100). The amount of this glycoprotein, which is barely detectable on the VLB-sensitive cells (CEM), appears to be related in part to the degree of resistance, up to approx 270-fold. Exposure of cells to Pronase for 45 to 60 min or growth of the cells for 2 days in tunicamycin, an inhibitor of glycoprotein synthesis, resulted in the absence of the resistance-associated glycoproteins, as determined by polyacrylamide gel electrophoresis of these treated cells after labeling the cells either with sodium [3H]borohydride or with [14C]- or [3H]glucosamine. Uptake studies with [3H]VLB revealed that CEM cells normally accumulated and retained more drug than did the CEM/VLB100 cells. While the tunicamycin or Pronase treatments slightly increased the uptake of drug by CEM cells, there was no enhanced uptake of [3H]VLB by the tunicamycin- or pronase-treated CEM/VLB100 cells, when compared with untreated controls, indicating that the loss of external surface glycoproteins did not render the resistant cells more 'leaky' to drug influx. Additionally, diminished drug r etention by the CEM/VLB100 cells was unaffected by these treatments. Moreover, when CEM/VLB100 cells were grown for 2 days in the presence of tunicamycin and several concentrations of VLB, no enhanced toxicity of VLB was noted. Other experiments with [14C]glucosamine and [3H]leucine revealed that treatment with tunicamycin did not affect the distribution of proteins in these cells. Taken together, these results permit the suggestion that the carbohydrate moiety of the cell surface resistance-associated glycoproteins does not mediate resistance to the alkaloid per se; however, a role for plasma membrane proteins cannot be ruled out at this time. (Author abstract) (19 Refs)

5. MEMBRANE GLYCOPEPTIDES FROM CHEMICALLY TRANSFORMED CELLS: COMPARISON BETWEEN MESENCHYMAL AND EPITHELIAL CELL LINES DERIVED FROM DIMETHYLNITROSAMINE-TREATED RAT KIDNEY.

Rachesky MH, Hard GC, Glick MC
(c/o Hard), Fels Res. Inst., Temple Univ. Sch. Medicine, 3420 North Broad St., Philadelphia, PA, 19140
Cancer Res; 42(1):39-43 1982

The membrane glycopeptides of normal and chemically transformed malignant cell lines derived from rat kidney were investigated by gel permeation chromatography. The cell lines, which were metabolically labeled with L-[5,6-3H]fucose or D-[6-3H]glucosamine, were derived from rats treated in vivo with a carcinogenic dose of dimethylnitrosamine to induce both mesenchymal and cortical epithelial tumors in the rat kidney. Among the cell lines examined were: (a) transformed lines of mesenchymal type derived from the rat kidney 2 to 24hr after the dose of carcinogen; (b) mesenchymal tumor cell lines obtained from the dimethylnitrosamine-induced mesenchymal tumors occurring at 7 to 10 mo; (c) a transformed epithelial cell line derived from rat kidney 48 hr after the carcinogenic dose; and (d) an epithelial cell line obtained from a renal adenocarcinoma induced at 10 mo by a dose of diethylnitrosamine. For control comparisons, populations of normal renal epithelium and mesenchyme were cultured selectively from untreated rats. The radioactive glycopeptides of the neoplastic epithelial cell lines (transformed and tumor) displayed a shift to more complex higher-mol-wt glycopeptides when compared with the normal epithelial counterparts. These results were similar to those reported previously for other cell types. In contrast, the neoplastic cell lines of mesenchymal origin lacked higher-mol-wt glycopeptides, displaying patterns that were identical with those of normal renal mesenchymal cells. It is suggested from these results that the membrane glycopeptide alterations characteristic of virus-transformed and other tumor cells are not detected by these techniques in all cell lines transformed by chemical agents. Furthermore, cell lineage within the same organ may play a role in the observed difference. (Author abstract) (22 Refs)

6. HETEROGENEITY IN SURFACE GLYCOPROTEINS OF MOUSE PERITONEAL MACROPHAGE POPULATIONS.

Kaplan G, Olstad R
Inst. Medical Biology, Univ. Tromso, Tromso, Norway
Exp Cell Res; 135(2):379-386 1981

Murine peritoneal macrophages were activated in vitro by repeated addition to the culture medium of cell-free tumor ascites fluid from a syngeneic methylcholanthrene-induced sarcoma. Cell morphology was studied by phase-contrast microscopy and scanning electron microscopy (SEM). The ascites-activated cells were extensively spread out on the glass surface and showed extensive membrane ruffling. To investigate the protein metabolism of the cells, total protein content and incorporation and turnover of [14C]glucosamine and [35S]methionine into TCA-precipitable macromolecules were analyzed. Activated cells had a total protein content 2-3x higher than that found in control cells. [35S]Methionine and [14C]glucosamine were incorporated into ascites-treated cells at a rate 2.7-3x higher than into control cells. Cell glycoproteins were lost with biphasic kinetics with a reduced half-life for both phases in the activated cells as compared to controls. Radiolabelled proteins were isolated from the plasma membrane by immunoprecipitation of trinitrobenzene sulfonic acid-derived cells and analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Whereas most of the protein peaks were the same in both cultures, the control cells had a single broad protein peak in the range of 100-110 K, whereas the ascites-activated cells had three peaks of 90, 100-110 and 135 K. The 135 K peak appeared to be a 'new' surface glycoprotein expressed in the activated but not in the control cells. (Author abstract) (29 Refs)

7. STUDIES ON THE LOCALIZATION OF THE GLYCOPROTEIN GP-2 WITHIN THE RENAL GLOMERULUS IN VIVO AND IN CULTURED KIDNEY CELL STRAINS IN VITRO.

Oberley TD, Chung AE, Murphy-Ullrich JE, Mosher DF
Dept. Pathology, Univ. Wisconsin Medical Sch., Madison, WI
J Histochem Cytochem; 29(11):1237-1242 1981

Positive staining for the glycoprotein GP-2 was demonstrated in the kidney glomerulus by use of the indirect peroxidase-labeled antibody technique. At the ultrastructural level, heaviest staining for GP-2 was demonstrated along the lamina rara externa and lamina rara interna of the glomerular and tubular basement membranes, demonstrating definite molecular organization for structures which appear amorphous even at the electron microscopic level. However, GP-2 was also present in the lamina densa of the glomerular basement membrane, but not of the tubular basement membrane. The staining for GP-2 is in contrast to the predominantly mesangial staining for fibronectin. Using the indirect immunoperoxidase techniques for kidney cells cultured in vitro, it was demonstrated that cell surfaces of specific subpopulations of g lomerular cells stained heavily for both fibronectin and GP-2, while renal medullary cells did not stain at all using specific antiserum to these molecules. GP-2 was present extracellularly and showed moderate staining in glomerular cell culture, while fibronectin showed heavy staining in this location. (Author abstract) (12 Refs)

8. HUMAN MALIGNANT MELANOMA CELL SURFACE GLYCOPROTEINS (MEETING ABSTRACT).

Portoukalian J, Dore JF
INSERM U 218, Lyon, France
Third European Workshop on Melanin Pigmentation, September 28-October 1, 1981, Prague, Czechoslovakia. Czechoslovak Medical Society 128 pp., 1981.

To investigate the possible origin of diversity in biological behavior of human malignant melanoma cell lines, a study of their cell surface glycoproteins was initiated, using melanoma cell lines at different passage levels. Cell surface glycoproteins were labelled with: 1) galactose oxidase, tritiated sodium borohydride, 2) neuraminidase, galactose oxidase, tritiated sodium borohydride, 3) periodic acid, tritiated sodium borohydride, and examined by polyacrylamide gel electrophoresis and fluorography. Whatever the cell line, there is an important radioactivity incorporation with technique 3, in glycoproteins of mol wt higher than 100K, meaning a great number of terminal sialic acids. However, galactose residues are accessible to galactose oxidase only after neuraminidase treatment, which indicates that they are masked by sialic acid. Labelled glycoproteins profiles of the cell lines are very different. With a cell line weakly tumorigenic in nude mice, most of the radioactivity is incorporated in a 110K mol wt glycoprotein; the other labelled glycoproteins having an higher mol wt with numerous galactose residues and few sialic acids. On the other hand, with a cell line highly tumorigenic on nude mice, we do not find a 110K glycoprotein but a 85K one, with numerous galactose residues and few sialic acids. Moreover, after a treatment with

a lysolecithin, these melanoma cells from microvesicles of which the glycoprotein composition was analyzed, in order to show the membranous nature of these vesicles. (no Refs)

9. HUMORAL IMMUNE RESPONSE IN MELANOMA AND GLIOMA PATIENTS: A SOLUBILIZED MELANOMA-MEMBRANE COMPONENT AS A TOOL FOR DETECTING CIRCULATING ANTIBODIES.

Birkmayer GD
Institut fur Zellbiologie, Goethestrasse 33, D-8000 Munchen 2, W. Germany
Cancer; 48(11):2399-2408 1981

In search of tumor-associated components in human melanomas and gliomas, plasma membranes were isolated from these tumors and compared morphologically, biochemically and immunologically with those from normal human brain. The preparations exhibited a comparable ultrastructure of empty vesicles of various form and size. The melanoma membrane consisted of at least 15 protein components and the glioma membrane consisted of at least 24 protein components, 4 of which were absent in the membrane of normal tissue, as revealed by polyacrylamide gel electrophoresis. The four tumor-associated polypeptides could be solubilized together with a few other membrane components by the non-ionic detergent Triton-X100 and were used as an antigen source for immunologic characterization of melanoma and glioma membranes. A rabbit antiserum prepared against these solubilized membrane components from melanoma and glioma membranes. A rabbit antiserum prepared against these solubilized membrane components from melanoma was absorbed with plasma membranes from a variety of normal tissue including RBC, lymphocytes, spleen, liver, lung, kidney, placenta and brain. The absorbed antiserum reacted with the membrane extract of all the 11 melanomas tested so far; in addition it crossreacted with an identically prepared extract from all the 15 gliomas tested. Membrane extracts from other tumors, such as kidney and mammary carcinoma, were negative in this respect. Furthermore, a rabbit antiserum prepared against the Triton extract from glioma membranes yielded after exhaustive absorption an immune precipitate not only with the specimens from the 15 other gliomas but also with those from the 11 melanomas. Electrophoretic analysis of the immune precipitate formed by the Ig fraction of the absorbed rabbit antiserum and the Triton-X100 extract from melanoma membranes exhibited six polypeptide bands, two of which appear antigen-derived as they were missing in the profile of the Ig pattern. A comparable electrophoretic pattern was obtained with the immune precipitate of the glioma antigen specimen. The mol wt of these two polypeptide was estimated on the basis of their electrophoretic mobility to be 55,000 and 10,000 daltons, respectively. The electrophoretic immunologic findings argue for a common membrane component in human melanoma and glioma patients. Using the Triton extract from melanoma membranes as an antigen source, precipitating antibodies could be detected in the Ig fraction of 16 of the 24 sera from melanoma patients and in that of 15 of the 23 glioma patients by means of counter-current electrophoresis. Sera from a variety of cancer patients with diseases other than melanoma or glioma as well as from patients without cancer were negative in this respect. (Author abstract) (37 Refs)

10. HUMAN MELANOMA-ASSOCIATED ANTIGENS; IMMUNOCHEMICAL AND MOLECULAR PROFILE.

Reisfeld RA, Galloway DR, Morgan AC, Walker LE, Bumol TF
Scripps Clinic and Res. Foundation, La Jolla, CA
Cancer Bull; 33(5):211-218 1981

Some of the immunochemical approaches taken in the authors' laboratory to isolate and characterize two human melanoma-associated antigens utilizing specific polyclonal and monoclonal antibodies are described. Analysis of cellular proteins released into the surrounding culture of several human melanoma cells lines indicated three major components detected by polyclonal antimelanoma xenoantisera with mol wt of 260K (260,000), 240K and 100K. The 240K component has been identified as fibronectin. The 100K component is found in spent media of both melanoma and carcinoma cell lines. The 100K and 260K dalton antigens which have affinity for specific lectins and metabolic radiolabeling with sugars strongly suggest they were glycoproteins. Other studies are reported on the use of monoclonal antibody for structural characterization, production of monoclonal antibody, subunit composition of 100K and 250K dalton melanoma antigens, and functional properties. It is apparent that the two melanoma-associated antigens of 100K and 250K dalton, defined immunochemically by monoclonal antibody, are likely to play a key role in the elicitation of host immune responses to malignant melanoma. Furthermore, it appears that changes in intracellular transport, glycosylation, and cell-surface expression of these two markers may be associated with neoplastic transformation. (12 Refs)

11. MONOCLONAL ANTIBODIES RECOGNIZING NORMAL HUMAN T LYMPHOCYTES AND MALIGNANT HUMAN B LYMPHOCYTES: A COMPARATIVE STUDY.

Martin PJ, Hansen JA, Siadak AW, Nowinski RC
Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
J Immunol; 127(5):1920-1923 1981

We have undertaken a direct comparison of eight different murine monoclonal antibodies that recognize antigens shared by human T cells and certain malignant B cells. Immune precipitation, lysostripping, and competitive binding experiments indicate that the antigenic determinants detected by antibodies Leu 1, T101, 17F12, SC1, A50, OKT1, and 10.2 are closely associated on the same molecular species and may in fact be identical. The antigenic determinant recognized by antibody 12.1, which has properties similar to the one detected by other antibodies, is present on a distinct cell-surface molecule. Our results emphasize the importance of a quantitative direct comparison of different monoclonal antibodies in distinguishing newly reported antibodies from those already described. Such a comparison can resolve apparent discrepancies that arise either from methodologic variations or from differences in antibody avidity. (Author abstract) (21 Refs)

12. STRUCTURAL FEATURES AND SELECTIVE EXPRESSION OF THREE LY-5+ CELL-SURFACE MOLECULES.

Tung JS, Scheid MP, Pierotti MA, Hammerling U, Boyse EA
Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY, 10021
Immunogenetics; 14(1/2):101-106 1981

Conventional Ly-5 alloantisera precipitate cell-surface molecules of three sizes: 200K, 205K, and 220K. In SDS-PAGE, the rat monoclonal antibody 74/8' precipitates the same three molecules from both Ly-5.1 and Ly-5.2 cells. Cleveland map-

ping of the three molecules, precipitated by reaction of conventional Ly-5 alloantisera or 74/8' monoclonal antibody with lysates of 125I-labeled cells, disclosed no differences among the three molecular forms, but markedly distinguished all three Ly-5.1 molecules from all three Ly-5.2 molecules. Each of the three molecular forms can be expressed independently of the other two by cloned culture lines of Ly-5+ cells of different hematopoietic lineage. All of the seven cloned lines tested expressed only one form. However, two of the seven uncloned culture lines tested, plasmacytoma MOPC-70A and the X.1 putative macrophage line which originated from an SJL tumor, yielded both 200K and 205K forms. (Author abstract) (16 Refs)

13. CHANGES IN THE PROTEINS OF TUMOR CELL PLASMA MEMBRANES OF MICE TREATED WITH POLYENE ANTIBIOTIC AND CYCLOPHOSPHAMIDE.

Oksman AIa, Shneider MA, Eremeeva AA, Karel'skaia NP, Tereshin IM
All-Union Res. Technological Inst. Antibiotics and Medicinal Enzymes, Leningrad, USSR
Antibiotiki; 26(9):692-694 1981

To evaluate the mechanism of potentiation of cyclophosphamide (CP) by polyene antibiotics, random-bred albino mice bearing Ehrlich ascites carcinoma were treated with CP (15 mg/kg, im) and/or with levorin (LV: 75 mg/kg, iv). The drugs were administered for 7 days, starting on day 2 of tumor transplantation. Inhibition of tumor growth in the animals treated with CP in combination with LV was 60-70% greater than that in the animals treated with CP alone. Electrophoretic analysis of plasma membranes of the tumor cells showed summation of the changes in the membrane protein composition induced by CP and by LV. (7 Refs) (In RUS)

14. ISOLATION OF A CHONDROITIN SULFATE PROTEOGLYCAN FROM A RAT YOLK SAC TUMOR AND IMMUNOCHEMICAL DEMONSTRATION OF ITS CELL SURFACE LOCALIZATION.

Oldberg A, Hayman EG, Ruoslahti E
La Jolla Cancer Res. Foundation, La Jolla, CA, 92037
J Biol Chem; 256(21):10847-10852 1981

A proteoglycan was isolated from ascites fluid produced by a rat yolk sac tumor. The glycosaminoglycan chains of the proteoglycan are all sensitive to digestion with chondroitinase ABC and about 90% are sensitive to chondroitinase AC. The proteoglycan contains 5% protein. Amino acid analysis revealed a high content of serine and glycine which together constitute 37% of the amino acids. Glutamic acid (glutamine) and aspartic acid (asparagine) are also abundant. Galactosamine accounts for 97% of the hexosamine and the remainder is glucosamine. These characteristics indicate that the glycosaminoglycan side chains of this proteoglycan are predominantly chondroitin sulfate with a smaller amount of dermatan sulfate. Antibodies to the proteoglycan were prepared by immunization of a rabbit with purified alkali-treated proteoglycan. Affinity-purified antibodies from the antiserum immunoprecipitated (35S)sulfate-labeled radioactiv ity from culture media of the yolk sac tumor cells known to contain chondroitin sulfate proteoglycan. This binding was inhibited by the intact purified proteoglycan but not by proteoglycan treated with papain, suggesting dependence of the reactivity of the antibodies on integrity of the protein part of the proteoglycan. Immunofluorescence of the cultured yolk sac tumor cells revealed localization of immune reactive proteoglycans at the cell surface. (Author abstract) (48 Refs)

15. COMPLEX CARBOHYDRATES AS DIFFERENTIATION MARKERS IN MALIGNANT BLOOD CELLS: GLYCOLIPIDS IN THE HUMAN LEUKEMIAS.

Klock JC, Macher BA, Lee WM, Greaves MJ, Lajtha L, Jasmin C, Bessis M, Ross D
1282 Moffitt Hosp., San Francisco, CA, 94143
Blood Cells; 7(2):247-255 1981

Cell surface glycolipids were studied in normal neutrophils and lymphocytes, chronic myelogenous and lymphocytic leukemia cells, and acute myelogenous and lymphoblastic leukemia cells. A few undifferentiated leukemias or leukemias of uncertain lineage were also studied. Glycolipids were extracted with thin layer and gas-liquid chromatography, gas chromatography-mass spectrometry, direct probe mass spectrometry, and enzyme treatment. Twenty glycolipids were identified and sequenced. Human WBC possess glycolipid patterns that distinguish them from other blood cells. Differences in glycolipids occur in WBC of myeloid and lymphoid types. Two main features emerge from study of glycolipids of human leukemic cells: (1) even though cells appear morphologically undifferentiated, all leukemias be classified as myeloid or lymphoid on the basis of their glycolipids; (2) complexity of cell surface glycolipids is positively correlated with their degree of morphological differentiation. No glycolipids were found in leukemic WBC which were not found in normal cells. Glycolipids may be useful as both cell- and differentiation-specific markers in human blood cells. Antigenic differences in human leukemic cells may partly be due to an altered distribution of complex carbohydrates at the cell surface. (11 Refs)

16. THE RELATIONSHIP BETWEEN PLASMA MEMBRANE LIPID COMPOSITION AND PHYSICAL-CHEMICAL PROPERTIES. II. EFFECT OF PHOSPHOLIPID FATTY ACID MODULATION ON PLASMA MEMBRANE PHYSICAL PROPERTIES AND ENZYMATIC ACTIVITIES.

Poon R, Richards JM, Clark WR
Jewish Hosp. St. Louis, 216 S. Kings Highway, St. Louis, MO, 63178
Biochim Biophys Acta; 649(1):58-66 1981

The fatty acid composition of plasma membrane phospholipids of the murine T lymphocyte tumor EL4 were systematically modified in an attempt to understand the relationship between lipid bilayer composition and plasma membrane physical and biological properties. Two plasma membrane enzyme activities, adenylate cyclase and ouabain-sensitive (Na+ + K+)-ATPase, were measured in normal and fatty acid- substituted EL4 plasma membrane fractions. The fatty acid effect on enzyme activities was similar to previously reported effects of fatty acids on cytotoxic T cell function. The activity of both enzymes was inhibited by saturated fatty acids, while unsaturated fatty acids had a moderate enhancing effect on both enzyme activities. Using two different nitroxide derivatives of stearic acid, the order parameter and approx rotational correlation times were calculated from ESR spectra of normal and fatty acid-modified plasma membranes. No significant difference was found in either parameter in these membranes. These results, in conjunction with earlier data from our laboratory and others, suggest that caution should be exercised in inferring changes in membrane 'fluidity' based on lipid modulation of biological membranes. (Author abstract) (38 Refs)

17. MEMBRANE CHOLESTEROL, TUMORIGENESIS, AND THE BIOCHEMICAL PHENOTYPE OF NEOPLASIA.

Coleman PS, Lavietes BB
Lab. Biochemistry, Dept. Biology, New York Univ., New York, NY
CRC Crit Rev Biochem; 11(4):341-393 1981

Arguments are given for the proposal that abnormalities in the biosynthetic regulation of lipids, particularly cholesterol, constitute early events in tumorigenesis and underlie many of the phenotypic hallmarks of malignant neoplasia. Information on the cholesterol molecule is presented and its proposed role as a lipid component of biological membranes is discussed. Some features of regulated cholesterol synthesis in normal vs neoplastic cells are highlighted; evidence that proliferating cells have a special requirement for sterol synthesis is documented. Views on the way in which aberrant regulation of cholesterol biosynthesis occurring early in tumorigenesis can lead directly to other documented characteristics of tumor cell metabolism, particularly those aspects concerning cellular respiration, are presented. The cancer-stricken host, in whom plasma membrane fragments are shed from the tumor as microvesicles containing tumor antigenic determinants, is considered. (300 Refs)

18. MEMBRANE ALTERATIONS IN FUSING AND PHORBOL ESTER-TREATED MYOGENIC CELLS.

Grove RI
University of South Florida, Tampa, FL, 33620
Diss Abstr Int [B]; 42(4):1431-B 1981

Cultured chick embryo myogenic cells differentiate and fuse to form multinucleated myotubes which become striated and acquire the potential to undergo Spontaneous Contractions. The diterpene tumor promoter, phorbol-12-myristate 13-acetate (PMA) inhibits myoblast fusion. The most rapid effects of this lipophilic compound occur in plasma membrane-associated processes, such as ion transport and cyclic nucleotide generation. Changes in specific membrane proteins have also been reported to occur in PMA-treated myogenic cells. In addition to the reported alterations in the levels of membrane protein s of known function it was possible that PMA extensively altered the composition and metabolism of other plasma membrane proteins whose functions are unknown. This hypothesis was tested using radiolabeled leucine and mannosamine precursors and electrophoretic analysis of membrane proteins on polyacrylamide gels. By 8 hr incubation PMA altered plasma membrane protein and sialoglycoprotein composition. Stimulation of diacylglycerol levels (2-fold) and stimulation of the rate of label incorporated into phosphatidic acid (2-fold), phosphatidylinositol (3-fold) and phosphatidylcholine (2-fold) were observed within 15-30 min of treatment with PMA. The increase in each of these lipids was shown to arise by synthesis from pre-existing lipids rather than by de novo synthesis. Using a double-label turnover technique and fatty acid analysis, it was shown that phosphatidylcholine is the most probable precursor of the PMA-induced diacylglycerol fraction. Subcellular fractionation of the PMA-stimulated cells suggested that the diacylglycerol was generated in the plasma membrane. Possible models for the mechanisms of PMA-dependent alterations in lipid metabolism and myoblast fusion are discussed. (no Refs)

MORPHOLOGICAL STUDIES

19. ADVANCES IN THE ULTRASTRUCTURE AND ELECTRON MICROSCOPY OF CANCER CELLS.

Hamilton DG
South African Inst. Medical Res., Post Office Box 1038, Johannesburg 2000, South Africa
S Afr Cancer Bull; 25(1):77-86 1981

An acquired genetic instability of cancer cells results in a heterogenous population which complicates ultrastructural studies. Tissue cell lines with well-defined and stable properties are required for most electron microscopy investigations. Cell surface pleomorphic villi occur on cancer cells, these villi are lost from the cell and contribute to cell membrane shedding with consequent loss of surface antigens. A necessary factor for invasion of tissues is the microfilament system which is reported to be increased, normal or decreased in cancer cells. The neoplastic cell nucleus shows nuclear membrane convolutions, nucleolar abnormalities and abnormal inclusions. The basal lamina can be present in invading cancers and may dictate the pattern of invasion. The increase in Weibel-Palade bodies in endothelial cells could be a morphologic marker of angiogenesis. (Author abstract) (32 Refs)

20. A PRELIMINARY ULTRASTRUCTURAL STUDY OF GASTRIC CANCER CELLS BY FREEZE-ETCHING TECHNIQUE.

Ying G, Li W, Cong Q, Yang J, Li X, Wang S, Li S, Zhao Y
Electron Microscopy Section, Inst. Basic Medicine, Hebei Medical Coll., Hebei Province, People's Republic of China
Zhonghua Yixue Zazhi; 60(4):229-231 1981

Results are described for an ultrastructural study of gastric cancer (9 cases), embryonic tissues of the stomach (4 cases) and normal stomach (4 cases). Tissues used were obtained by bi opsy under fibrogastroscopy or from surgery, and were prepared by the freeze-etching technic. Transmission electron microscopy examination revealed numerous abnormal changes in the cancerous tissue included changes in the microvilli, cell membrane, gap junctions, Golgi complexes and nuclear pores. (10 Refs) (In CHI)

21. ELECTRON-MICROSCOPICAL INVESTIGATIONS ON MELANOMA-CELLS AFTER HYPERTHERMIC CYTOSTATIC PERFUSION (MEETING ABSTRACT).

Ishii M, Paul E, Aigner K, Illig L
Zentrum fur Dermatologie, Justus-Liebig-Universitat, Giessen, W. Germany
Third European Workshop on Melanin Pigmentation, September 28-October 1, 1981, Prague, Czechoslovakia. Czechoslovak Medical Society 128 pp., 1981.

Electron-microscopic investigations were performed in three clinically evident primary tumors (superficial spreading melanoma) and in multiple cutaneous in-transit metastases of malignant melanoma after hyperthermic cytostatic perfusion. Indications of perfusion-induced cellular damage included: vacuolization of mitochondria, alteration of the nuclear shape with increased invagination, nuclear inclusions, and loss of nuclear membrane with dissolution of the normal chromatin structure. Cellular inclusions, which consist almost exclusively of aggregated melanosomes, are frequently observed, as well as loss of cell membrane and dissolution of cell structure. It appears that cellular damage does not occur immediately after the perfusion and that not all the tumor cells are affected. In the case of me-

tastases, as early as two wk after the perfusion, giant cells are frequently observed. In primary tumors, the cellular damage is most evident in the deepest, most invasive tumor parts. In metastases, the most striking cellular changes are observed in the center of the metastatic nodules. (no Refs)

22. SEQUENTIAL, LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF HEPATOCELLULAR CARCINOMA INDUCED BY AROCLOR 1260 (MEETING ABSTRACT).

Weltman RH, Norback DH
William S. Middleton Memorial Veterans' Hospital and University of, Wisconsin Madison, WI
Toxicologist; 1(1):65 1981

Progressive hypertrophic (HT), hyperplastic (HP) and neoplastic (N) growth was observed in female Sprague-Dawley rats continuously exposed to a diet containing 100 ppm, followed by 50 ppm, Aroclor 1260. The liver of control rats showed no gross, lobular or subcellular changes. Central cell hypertrophy was present following 1 mo of Aroclor exposure. The eosinophilic HT cell contained increased smooth endoplasmic reticulum (ER), cytoplasmic vacuoles (CV), lipid droplets (LD) and a hyperchromatic nucleus with a large nucleolus. Foci and areas of HP cells were observed in all livers following 9 mo of Aroclor exposure. In addition to HT cell cytoplasmic characteristics, HP cells possessed increased nuclear chromatin granules, LD and pseudo inclusions. Scanning electron microscopy (SEM) of HP cells revealed disordered cellular arrangement and dilated, tortuous canalicular channels. Firm, elevated, tan, 0.5-1 cm N growths were observed in all livers examined after 15 mo of Aroclor exposure. Four-cell-thick trabeculae, acinar arrangements, hemorrhage, necrosis and a few mitoses indicate that the large N growths are hepatocellular carcinoma. In addition to the cellular features of HP cells, N cells contained nucleolar segregation, concentric membrane arrays, short stacks and single strands of rough ER, and abundant mono- and polyribosomes. Rough ER encircled mitochondria and peroxisomes. In pericanalicular regions microvilli-lined CV and abundant filaments were observed. SEM of N tissue showed disordered cellular arrangement, dendritic canalicular channels and many surface particles. (no Refs)

MEMBRANE RECEPTOR/ BINDING SITES

23. SURFACE MEMBRANE CHARACTERISTICS AND CYTOCHEMISTRY OF THE ABNORMAL CELLS IN NONLYMPHOCYTIC LEUKEMIA.

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Nippon Naika Gakkai Zasshi; 70(4):553-564 1981

The surface membrane characteristics and cytochemistry of the leukemia cells were determined in 20 patients with acute myelogenous leukemia (AML), 2 with acute premyelogenous leukemia (APL), 3 with chronic myelogenous leukemia with malignant change (CMLC), 2 with acute monocytic leukemia (AMoL), 12 with acute-subacute myelogenous monocytic leukemia (MMoL) and with chronic myelogenous monocytic leukemia (CMMoL). The cells were examined for Fcgamma, Fcmu, C3b and *C3d receptors* by the percentage of rosette-forming cells by means of a microtest plate. Human group AB serum and AKR mouse serum were used as complement. Phagocytosis of the leukemia cells was also determined. Since most of the patients having cytoplasmic granules and peroxidase (PO) among the patients with AML and APL had Fcgamma or C3 receptors, the receptors, granules and PO were considered to appear simultaneously. CML (with malignant change) patients had receptors although only a small amount of enzymes was expressed. All of the patients with AMoL and MMoL had several receptors, and phagocytosis was also detected in all of the patients. Cytochemically, 5 of the 18 patients with AMoL, MMoL or CMMoL completely lacked alpha-naphthyl butyrate esterase in their monocytic leukemia cells, and 7 of the patients partially lacked this enzyme. Phagocytosis was detected in all of the patients, even those who lacked this enzyme. Therefore, the finding of immunological phagocytosis was considered valuable as evidence that a monocytic element constituted the leukemia cell. (20 Refs) (In JPN)

24. IN-VITRO RECONSTITUTION OF BETA-ADRENERGIC RESPONSE IN HUMAN LEUKEMIA CELLS BY C ELL-CELl AND MEMBRANE-CELL FUSION (MEETING ABSTRACT).

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First Medical Clinic, University of Vienna, Lazarettgasse 14, A-1090 Vienna, Austria
Exp Hematol; 9(Suppl9):85 1981

For the first time, Orly and Schramm succeeded in the functional transplantation of hormone receptors from one cell to another. Shortly afterwards, we reported on the complementation of the beta-receptor/adenylate cyclase system not only by fusion of intact cells but also by using membrane fragments as beta-receptor donors. Heterokaryons formed by fusion or adenylate cyclase-deficient S49 mouse lymphoma cells (AC-) and beta-adrenergic receptor-deficient B82 mouse fibroblasts displayed a catecholamine-sensitive adenylate cyclase activity. Comparable results were obtained when B82 fibroblasts were fused with membrane particles from the AC- variant. For standardization of the experimental conditions, human leukemic cell lines grown continuously in culture were chosen. The cell lines were screened for adenylate cyclase activity and superficial beta-adrenergic receptor density. Three types of cell lines expressing differences in the receptor/enzyme system evolved from these studies: Group I: Cells which exhibited a high number of beta-receptors and a correspondingly strong adenylate cyclase stimulation by isoproterenol, a potent beta-adrenergic agonist. Group 2: Cells which exhibited an equally high beta-receptor density but completely lacked adenylate cyclase response to isoproterenol. Group 3: Cells which neither possessed a remarkable number of beta-receptors nor showed any adenylate cyclase response to isoproterenol, despite a pronounced, response of the enzyme to prostaglandin E1. Cells from groups 2 and 3 were chosen for experiments with the cell-cell and membrane fusion technique. It could be demonstrated that the polyethyleneglycol-induced fusion of beta-receptor deficient cells (group 3) with intact cells or membrane particles from beta-receptor replete cells (group 2) resulted in the reconstitution of the beta-adrenergic response in the fusion products. This response could be specifically blocked by the beta-adrenergic antagonist propranolol. (2 Refs)

25. SPECIFIC BINDING OF 125I-INSULIN IN PLASMA MEMBRANES OF FAT CELLS IN PATIENTS WITH PHEOCHROMOCYTOMA.

Bezdrobnyi IuV, Evdokimova NIu, Efimov AS, Komissarenko IV, Demchenko NP
Diabetes Dept., Inst. Endocrinology and Metabolism, Kiev, USSR
Probl Endokrinol (Mosk); 27(5):38-42 1981

The insulin receptors of fat cell membranes were studied in seven pheochromocytoma patients. The membranes were isolated from surgery specimens of paranephric or mesenteric fat and were incubated with labeled and nonlabeled insulin. The pheochromocytoma patients showed a significant decrease in specific binding of the 125I-insulin. The decrease was due to reduction of insulin-binding sites and dec reased affinity for insulin. The changes in the state of the insulin receptors were associated with an increased level of catecholamines. (14 Refs) (In RUS)

26. EVIDENCE FOR A FOLIC ACID BINDING PROTEIN IN HUMAN CELL MEMBRANE.

Corrocher R, Pachor ML, Bambara LM, De Sandre G
Instituto di Patologia Speciale Medica II, Policlinico di Borgo Roma, I-37134 Verona, Italy
Acta Haematol (Basel); 66(3):202-209 1981

Cell membranes were prepared by sucrose discontinuous gradient from human liver and human peripheral WBC and RBC and from circulating WBC from patients with chronic granulocytic leukemia (CGL) and acute myeloblastic leukemia (AML). The membrane preparations from liver and from leukemic WBC were shown to bind tritiated folic acid. The membranes from normal WBC and RBC did not show this binding capacity. The membrane preparation from liver and CGL WBC showed two peaks of binding eluting with proteins from Sephadex G-200. However, protein extracts of these membrane preparations showed only a single peak for labelled folic acid, eluting near but just after albumin. The binding capacity of the membranes for folic acid was partially inhibited by reduced folate analogues. It is concluded that the liver plasma cell membrane and the membranes of myeloblasts in AML and circulating WBC in CGL contain a binding protein for folic acid which may be concerned in the transport of folates into these cells. (Author abstract) (22 Refs)

27. EVIDENCE FOR THE ASSOCIATION OF TWO CELL SURFACE GLYCOPROTEINS OF 13762 MAMMARY ASCITES TUMOR CELLS: CONCANAVALIN A-INDUCED REDISTRIBUTION OF PEANUT AGGLUTININ-BINDING PROTEINS.

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Exp Cell Res; 135(2):418-424 1981

The behavior of the cell surface concanavalin A (con A) receptors and of peanut agglutinin (PNA) receptors on the MAT-B1 ascites subline of the 13762 rat mammary adenocarcinoma was examined using fluorescein-labeled conA and PNA. ASGP-1, the major glucosamine-containing glycoprotein of these ascites cells, is the only PNA-binding protein observed by dodecyl sulfate electrophoresis. ASGP-2, the second most prominent component after glucosamine labeling, is the most abundant conA-binding protein. These two glycoproteins were previously shown to be associated as a complex in detergent extracts of the cells. ConA-binding proteins, upon incubation with fluorescein-labeled conA (FITC-conA), redistribute on the cell surface into small and large aggregates similar, but not identical, to those seen in 'patching' and 'capping' experiments with lymphocytes. PNA-binding proteins failed to redistribute during incubation with fluorescein-labeled PNA (FITC-PNA) and appeared in a diffusely stained pattern around the circumference of the cells. However, when cells were treated with unlabeled conA followed by FITC-PNA, or with FITC-PNA followed by unlabeled conA, there was marked redistribution of the FITC-PNA. These results indicate that ASGP-1 redistributes in response to the movement of conA-binding proteins, and supports our hypothesis that ASGP-1 and ASGP-2 are associated on the plasma membrane at the cell surface as well as in detergent extracts. (Author abstract) (22 Refs)

28. CHANGES IN SURFACE ARCHITECTURE DURING MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION AS DETECTED BY LECTIN BINDING AND AGGLUTINATION.

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Biochim Biophys Acta; 649(1):105-112 1981

Cell surface alterations occurred during murine erythroleukemia cell (clone 745) differentiation that were detected by both agglutination and lectin binding. Agglutination of erythroleukemia cells were produced by wheat germ agglutinin, whereas, concanavalin A, ricin, soybean agglutinin and fucose-binding protein were either ineffective or much less efficacious. Treatment of leukemia cells with the inducer of erythroid differentiation dimethylsulfoxide (DMSO) caused a progressive accumulation of Hb-containing cells in culture and a decrease in the rate of agglutination by wheat germ agglutinin, which began at 24 hr after exposure to the polar solvent, reached a nadir at 48 hr, and remained essentially constant thereafter. The binding of radioactive wheat germ agglutinin by untreated control erythroleukemia cells increased with time in culture, reaching a max value at 48 hr, and decreased progressively thereafter. Although an increase in 3H-labeled wheat germ agglutinin binding also occurred in DMSO-treated cells, the level bound was significantly lower than that observed in control cells at 24-96 hr. The treatment of erythroleukemia cells with various concentrations of DMSO resulted in a decrease in the number of wheat germ agglutinin *receptor sites*. Other inducers of differentiation (ie, dimethylformamide, bis(acetyl)diaminopentane) also inhibited the rate of wheat germ agglutinin-induced agglutination of erythroleukemia cells while, in contrast, the inducer tetramethylurea did not. These studies indicate that membrane changes occur during differentiation and suggest that there may be more than one mechanism involved in the initiation of maturation which ultimately leads to the common pathway of erythroid development. (Author abstract) (39 Refs)

29. RHODAMINE ISOTHIOCYANATE COUPLED PEANUT LECTIN FOR QUANTITATIVE STUDIES OF D-GALACTOSYL RECEPTORS OF NEUROBLASTOMA CELLS.

Caron M, Deugnier MA, Albe X, Bisconte JC, Faure A
Lab. Immunochemistry, Quantitative Neurobiology, U.E.R. Biomedicale, 74 rue Marcel Cachin, F-93012 Bobigny Cedex, France
Experientia; 37(11):1154-1157 1981

Rhodamine-peanut agglutinin conjugate has been obtained without alteration of the lectin activity. This conjugate interacts specifically with terminal D-galactosyl glycoconjugates. Such receptors are found on neuroblastoma differentiated or

undifferentiated cell membranes. The density and distribution of these galactosyl sites are different after neuraminidase treatment. (Author abstract) (15 Refs)

TRANSPORT STUDIES

30. HEXOSE TRANSPORT IN HYBRIDS BETWEEN MALIGNANT AND NORMAL CELLS.

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Nature; 294(5838):232-235 1981

The kinetic parameters of hexose uptake were measured in matched pairs of hybrids between malignant and normal cells. Each pair consisted of a hybrid in which malignancy was initially suppressed and a segregant tumor derived from it. Comparisons were also made between tumor cells and non-tumorigenic derivatives selected from the tumor cell populations in vitro. Without exception, malignancy, as defined by the ability of the cell to grow progressively in vivo, was found to be linked to a systematic decrease in the Michaelis constant of the hexose transport system. (Author abstract) (22 Refs)

31. HEXOSE TRANSPORT IN EPSTEIN-BARR VIRUS (EBV) NEGATIVE LYMPHOMA LINES AND THEIR EBV CONVERTED VIRUS GENOME CARRYING SUBLINES.

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Blut; 43(5):297-305 1981

The uptake of (3H)-glucosamine (3H-G) and (3H)-2-deoxy-D-glucose (3H-DDG) into BJAB and Ramos cell lines and their Epstein-Barr Virus (EBV)-converted sublines was analyzed. 3H-G uptake was enhanced in the EBV-positive BJAB-B95 subline compared to the EBV-parental BJAB line; uptake was linear in both control and EBV-converted cells from 2-20 min, at 37 C. 3H-DDG was also taken up by BJAB cells in a linear manner over 20 min at a similar rate, indicating that the virus has a direct or indirect effect on the membrane glucose carrier. This was further underlined by the observed competition of glucose with 2DDG and by the chase of 3H-DDG through glucose. EBV-converted cells took up both sugars at an increased rate compared with the EBV-negative parental lines. EBV-conversion produced an increase of 3H-DDG uptake of 170% in BJAB-B95-8 and BJAB-P3HR-I, and 120-130% in the case of converted Ramos cells. The 3H-G uptake was increased by 120% and 220% in converted BJAB and by approx 160% in converted Ramos cells. (37 Refs)

32. CHARACTERIZATION AND PARTIAL PURIFICATION OF AMINO ACID TRANSPORT SYSTEMS FROM PLASMA MEMBRANES OF EHRLICH CELLS.

Johnson PA
McGill University, Montreal, Canada
Diss Abstr Int [B]; 42(4):1433-B 1981

Identification of amino acid transport protein(s) in Ehrlich plasma membranes was approached by selective elution of protein from the plasma membranes to enrich the transport activity(ies) in the residual pellet. The original plasma membranes were extracted with 0.25% Triton X-100. A second extraction with 2,3-dimethylmaleic anhydride (2 mg/ml) followed. Extracted vesicles maintained sodium-coupled amino acid transport. Accelerated amino acid exchange diffusion was lost after 2,3-dimethylmaleic anhydride extraction. The extracted vesicles contained 25% of the original protein and 73% of the original phospholipid associated with the native vesicles, and were enriched two-fold in carbohydrate residues. Intact Ehrlich cells treated with hydrolytic enzymes to determine which components on the external cell surface are required for active amino acid transport. Trypsin treatment of Ehrlich cells reduced sodium-coupled amino acid transport, which could be restored by incubation with 8% serum. The decrease in active amino acid transport appears to result from an alteration in membrane permeability caused by deoxyribonuclease-sensitive material. The latter is released from cells during treatment. Removal of neuraminidase-sensitive sialic acid from the surface of Ehrlich cells has no effect on sodium-coupled amino acid transport. (no Refs)

33. STIMULATION BY PHOSPHATIDIC ACID OF CALCIUM INFLUX AND CYCLIC GMP SYNTHESIS IN NEUROBLASTOMA CELLS.

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J Biol Chem; 256(21):10945-10948 1981

Phosphatidic acid added to the medium markedly elevated intracellular cyclic GMP content in cultured neuroblastoma N1E 115 cells. There was a significant elevation of cyclic GMP with 1 ug/ml and a max (70-fold) elevation with 100 ug/ml of phosphatidic acid. Other natural phospholipids did not increase, or increased only slightly the cyclic GMP content in the cells. The elevation of cyclic GMP content by phosphatidic acid was absolutely dependent on extracellular calcium. Phosphatidic acid stimulated the influx of calcium into neuroblastoma cells two- to five-fold. The pattern of the calcium influx induced by phosphatidic acid was comparable to that of cyclic GMP elevation. The stimulation of calcium influx by phosphatidic acid was also observed in cultured heart cells, indicating that phosphatidic acid acts as a calcium ionophore or opens a specific calcium-gate in a variety of cell membranes. Treatment of neuroblastoma cells with phospholipase C increased 32Pi labeling of phosphatidic acid, stimulated the influx of calcium, and elevated the cyclic GMP content in the cells. Thus exogenous as well as endogenous phosphatidic acid stimulates the translocation of calcium across cell membranes and, as a consequence, induces the synthesis of cyclic GMP in the neuroblastoma cells. (Author abstract) (32 Refs)

34. RUTHENIUM RED-INSENSITIVE CALCIUM TRANSPORT IN ASCITES-SARCOMA 180/TG CELLS.

Bygrave FL, Anderson TA
Dept. Biochemistry, Faculty Science, Australian Natl. Univ., Canberra, A.C.T. 2600, Australia
Biochem J; 200(2):343-348 1981

Ruthenium Red-insensitive Ca++ transport in the mouse ascites sarcoma 180/TG is enriched in a 'heavy' microsomal fraction (microsomes) sedimented at 35,000 g for 20 min. The subcellular distribution of this Ca++ transport differed from that of Ruthenium Red-sensitive Ca++ transport and (Na+ + K+)-dependent ATPase activity, but was similar to that of glucose 6-phosphatase. The affinity of this transport system for 'free' Ca++ is high (Km approx 6 uM) and that for MgATP somewhat lower (Km approx 100 uM). Ca++ transport by the tumor microsomes, by contrast with that by liver micro-

somes, was greatly stimulated by low concentrations of Pi. Although incubation of intact ascites cells with glucagon led to an increase in intracellular cyclic AMP, no stable increase in the initial rate of Ca++ transport in the subsequently isolated 'heavy' microsomes could be detected as in similar experiments carried out previously with rat liver cells. Reconstitution experiments suggest that a deficiency exists in the tumor microsomal membrane such as an action of glucagon that is normally present in rat liver microsomes is not evoked. (Author abstract) (20 Refs)

35. BINDING OF NITROBENZYLTHIOINOSINE TO HIGH-AFFINITY SITES ON THE NUCLEOSIDE-TRANSPORT MECHANISM OF HELA CELLS.

Dahlig-Harley E, Eilam Y, Paterson AR, Cass CE
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Biochem J; 200(2):295-305 1981

Nitrobenzylthioinosine (NBMPR) binds reversibly, but with high affinity (Kd 0.1-1.2 nM), to inhibitory sites on nucleoside-transport elements of the plasma membrane in a variety of animal cells. The present study explored relationships in HeLa cells between NBMPR binding and inhibition of uridine transport. The Km value for inward transport of uridine by HeLa cells in both suspension and monolayer culture was about 0.1 mM. The affinity of the transport-inhibitory sites for uridine (Kd 1.7 mM), inosine (Kd 0.4 mM) and other nucleoside permeants was low relative to that for NBMPR. The pyrimidine homologue of NBMPR, nitrobenzylthiouridine, also exhibited low affinity for the NBMPR-binding sites. Pretreatment of HeLa cells with p-chloromercuribenzene sulfonate (p-CMBS) or N-ethylmaleimide (NEM) decreased binding of NBMPR to its high-affinity sites and inhibited uridine transport, indicating the presence of thiol groups essential to both processes. NEM, a more penetrable reagent than p-CMBS, inhibited binding and transport at much lower concentrations than the latter compound. Pretreatment of cells with concentrations of p-CMBS that alone had no effect on either NBMPR binding or uridine transport increased the sensitivity of transport to NBMPR inhibition and changed the shape of the NBMPR concentration-effect curve, suggesting synergistic inhibition of uridine-transport activity by these two agents. (Author abstract) (29 Refs)

36. REDUCED GLUTATHIONE TRANSPORT INTO INVERTED RED CELL MEMBRANE VESICLES IN THE INVESTIGATION OF RAISED INTRACELLULAR LEVELS OF RED CELL GSH IN ACUTE LEUKAEMIA (MEETING ABSTRACT).

Lieberman JE, Gordon-Smith EC
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Clin Sci; 61(3):17P 1981

Elevated levels of red cell reduced glutathione (GSH) have been reported for patients with acute and chronic leukemia. Blume et al (1973) have suggested that the raised Intracellular levels of GSH may in part be due to increased activity of GSH synthetase. While the fate of normal red cell GSH has not been fully elucidated it has been suggested that outward transport of oxidized glutathione (GSSG) contributes to the turnover of GSH. We found that the elevated levels of GSH in patients' red cells are not due to a change in the GSH/GSSG ratio. There is no correlation between PCV and total blood GSH. The possibility that GSSG transport was impaired led us to attempt to measure GSSG flux from these red cells but we were unable to confirm previous reports of this flux. To test whether GSH could pass through the red cell membrane, inside out membrane vesicles (1.0s) were prepared from pure red cell suspensions by the method of Steck et al (1973). When these 1.0s were incubated with [14C]-GSH, the GSH was found to diffuse into the 1.0s. This diffusion showed rate limiting characteristics with increasing substrate concentration indicating a carrier mediated mechanism. Mg++ and ATP greatly increased the rate of flux but Mg++ or ATP alone had no effect. Ca++, Na+, K+ and ouabain did not affect the GSH flux. The 1.0 status of the vesicles was confirmed by testing for Mg++ATP stimulated uptake of [45]-Ca++. When right side out vesicles were used, Mg++ATP stimulated uptake of [45]-Ca++ and GSH was not obtained. These studies indicate the presence of a GSH, Mg++ dependent membrane bound transport ATPase in red cells. The rate of transport could account for part of the GSH turnover in red cells. Impairment of this transport may play a role in the raised levels of GSH found in acute leukemia. (2 Refs)

37. CARRIER-DEPENDENT AND CARRIER-INDEPENDENT TRANSPORT OF ANTI-CANCER ALKYLATING AGENTS.

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Nature; 294(5838):281-283 1981

Alkylating agents were found to comprise two groups, those whose uptake into the cell depends on a membrane transport system, and those not so dependent. Phytohemagglutinin (PHA) was used in an assay to induce proliferation in tissue culture. Uptake of 3H-thymidine in human peripheral T-lymphocytes was very low before PHA stimulation but increased steadily with time in the 18 hr after its addition. In this assay unstimulated lymphocytes are considered a 'resting' cell population, and after 18 hr exposure to PHA they are considered to be actively proliferating. To test effects of alkylating agents on these two populations of cells, various drug concentrations were added either 60 min before or 18 hr after PHA stimulation. The cytotoxicity of various alkylating agents and x-rays was determined quantitatively, using a series of increasing drug concentrations to obtain reasonably complete (2-3 log death) survival curves. Two clearly distinguished survival patterns emerged. The first, termed carrier dependent, is characterized by dose-dependent cell death but with a substantially greater shoulder on the survival curve of resting cells. Melphalan, nitrogen mustard, and cis-dichlorodiammineplatinum gave this pattern. In the second pattern, termed carrier-independent toxicity, drugs gave identical surviving curves for resting and cycling cells, and all are highly lipid-soluble. Drugs in this pattern include the nitrosoureas, procarbazine, busulfan, mitomycin C and ACNU [1-(4-amino-2-methyl-pyrimidin-5-yl)-methyl-3-(2 chloroethyl)-3-(nitrosourea)]. (52 Refs)

38. ENTRY AND DISTRIBUTION OF CHLORPROMAZINE AND VINBLASTINE INTO HUMAN ERYTHROCYTES DURING ENDOCYTOSIS.

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Div. Hematology, Dept. Medicine, Stanford Univ., Stanford, CA, 94305
Proc Soc Exp Biol Med; 168(2):159-167 1981

Entry and distribution of 3H-labeled vinblastine (VLB) and chlorpromazine (CPZ) into human RBC during endocytosis was investigated. Drug-induced endocytosis was measured by phase microscopy and quantitative radioiostopic method. RBC suspended in Hanks' or sucrose medium were centrifuged,

mixed with prewarmed CPZ (577 nanomole/ml) and VLB (573 nanomole/ml), vortexed, diluted with phosphate buffered saline, and centrifuged. Radioisotopic determination was performed on the supernatant and RBC fractions, and cytosol and membrane subfractions. Results showed CPZ entry into RBC complete by 0 time while VLB entry into RBC took place during the first 5 min of incubation. Values for CPZ content of RBC and their membranes suspended in either Hanks' or sodium lactate medium were similar with 80-90% of CPZ found in cytosol, and 10-20% found in the plasma membrane. VLB produced constant values in the membrane fraction (1-11%) in both Hanks' and sodium lactate medium. Sucrose produced no regular alteration in CPZ incorporation or distribution within RBC despite a three to fivefold increase in CPZ endocytosis in this media. Sucrose c learly reduced the rate of VLB uptake into RBC but not the proportion of VLB recovered in the membrane fraction. Apparently the interaction of the drug-induced membrane and cytosolic events lead to endocytosis. Endocytosis induced by CPZ does not require ATP, is not affected by Ca++, and is enhanced by sucrose medium. Endocytosis induced by VLB requires ATP, is inhibited by Ca++ and sucrose medium. Effects of sodium lactate and isotonic sucrose media on these interactions remain to be determined. (19 Refs)

39. ACTIVE TRANSPORT OF 5-FLUOROURACIL AND ITS ENERGY COUPLING IN EHRLICH ASCITES TUMOR CELLS.

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J Biochem (Tokyo); 90(3):635-642 1981

Ehrlich cells accumulated 5-fluorouracil (FUra) and its metabolites in the acid-soluble pool to a level 5x that in the medium in the presence of glucose after 60 min of incubation at 37 C. FUra taken up in 10 min under the same conditions was almost wholly present as the non-metabolized form, and the intracellular level of non-metabolized form was 2 and 3x the extracellular concentration after 30 and 60 min of incubation, respectively. The uptake of FUra was dependent on temperature with an optimum at 42 C. The initial rate of FUra uptake showed saturation kinetics with a Km for FUra of 0.63 mM. Pyrimidine analogs inhibited FUra transport competitively, and sulfhydryl reagents also inhibited the transport. These results indicate that presence of a carrier system specific for FUra, which catalyzes an active transport of FUra into Ehrlich cells. Concentrative uptake of FUra in the presence of glucose was inhibited by glycolytic inhibitors, but not by the addition of mitochondrial electron transfer inhibitors and uncouplers. The uptake rate of FUra was found to be dependent on cellular ATP concentration in the presence and absence of extracellular Na+. A decrease in the uptake rate at lower intracellular ATP level was due to a decrease in the Vmax value for FUra and the half-max velocity for the uptake was observed at 2.4 mM ATP regardless of the presence of extracellular Na+. Although FUra was accumulated in the absence of extracellular Na+ in Ehrlich cells, the initial rate of FUra uptake was increased 1.3-fold in the presence of an Na+ gradient (out greater than in) due to an increase in Vmax for FUra and the half-max velocity for Na+-dependent uptake was observed at 21 mM extracellular Na+ concentration. These results suggest that active transport of FUra into Ehrlich cells may be directly coupled to intracellular ATP. (Author abstract) (52 Refs)

MEMBRANE BIOGENESIS AND METABOLISM

40. DIFFERENCES IN SYNTHESIS OF MEMBRANE PROTEINS BY LEUKEMIC CELLS FROM SPLEEN AND PERIPHERAL BLOOD INDICATE DISTINCT SUBSETS OF MALIGNANT CELLS IN A PATIENT WITH PROLYMPHOCYTIC LEUKEMIA.

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(c/o Humphreys) Dept. Pharmacology, Univ. Massachusetts Medical Sch., 55 Lake Ave. North, Worcester, MA, 01605

Am J Hematol; 11(3):261-270 1981

Morphological and biochemical differences were demonstrated between prolymphocytic leukemia cells obtained from the spleen and peripheral blood of one patient. Peripheral blood prolymphocytes had consistently smaller nuclear-cytoplasmic ratios than did splenic prolymphocytes. Percoll gradient-purified prolymphocytes from the spleen synthesized abundant amounts of some membrane proteins which were hardly expressed by peripheral blood prolymphocytes. Peripheral blood prolymphocytes did not change their expression of membrane proteins during three days in culture. These findings are consistent with the view that prolymphocytic leukemia cells from the spleen exist, on the av, at an earlier stage of differentiation than do circulating leukemic cells, and that peripheral blood leukemic cells are frozen at a specific phase of differentiation. (Author abstract) (26 Refs)

41. CELL JUNCTION AND CYCLIC AMP: III. PROMOTION OF JUNCTIONAL MEMBRANE PERMEABILITY AND JUNCTIONAL MEMBRANE PARTICLES IN A JUNCTION-DEFICIENT CELL TYPE.

Azarnia R, Dahl G, Loewenstein WR

Dept. Physiology and Biophysics, Univ. Miami, Sch. Medicine, Miami, FL, 33101

J Membrane Biol; 63(1/2):133-146 1981

The effect of cyclic nucleotides on promotion of junctional membrane permeability and differentiation was studied in C1-1D, a strain of murine cancer cells lacking permeable junctions in confluent culture (CC). CC were exposed to 1 mM concentrations of cyclic AMP (cAMP), dibutyryl cAMP (db-cAMP), db-cAMP and caffeine, or cholera toxin. In each case permeable intercellular junctions were formed, and cells transferred carboxyfluorescein to each other. The effects were complete within hours. Similar effects were produced in non-CC and in cultures that were starved for serum, a condition the caused increased cAMP levels. In non-CC, formation of junctions and differentiation of junction membranes was inversely proportional to cell density. Cells in low-density cultures were able to form junctions with human Lesch-Nyhan or murine 3T3-Balb C cells. Electrical coupling of cells was not inhibited by the cytokinesis inhibitor cytochalasin B (1 ug/ml). The protein synthesis inhibitor cycloheximide prevented formation of recognizable gap junctions and tight junctions in CC treated with db-cAMP. (37 Refs)

CELL ADHESION

42. FIBRONECTIN AND GLIAL FIBRILLARY ACIDIC PROTEIN EXPRESSION IN NORMAL HUMAN BRAIN AND ANAPLASTIC HUMAN GLIOMAS.

Jones TR, Ruoslahti E, Schold SC, Bigner DD
(c/o Bigner) Dept. Pathology, Duke Univ. Medical Center, Durham, NC, 27710
Cancer Res; 42(1):168-177 1982

The expression of fibronectin (FN), an extracellular matrix glycoprotein with adhesive and opsonic properties, was examined in normal and neoplastic human CNS tissues. In both normal adult and fetal brain, FN expression, as detected by immunohistochemistry, was restricted to the vasculature. Neurons and glia did not stain. FN expression in 9 of 10 gliomas examined was qualitatively similar to that in normal fetal and adult brain in that it was confined to the blood vessels. Nevertheless, in one case, there was interstitial expression of FN. Four tumors exhibited varying degrees of fibroblastic overgrowth in which cells containing FN were detected. Frozen sections of eight athymic mouse-borne anaplastic human gliomas were stained simultaneously by a double-label fluorescein-rhodamine fluorescence technique for both FN and glial fibrillary acidic protein (GFAP), a glial and glioma marker. Seven of eight tumors expressed FN, seven of eight expressed GFAP, and six of eight expressed both. FN was detected as fine fibrils and specks between cells while GFAP presented as a homogeneous intracellular fluorescence. In many areas of the tumors, groups of cells expressed both FN and GFAP. Eleven human glioma-derived cell lines were examined for both FN and GFAP expression. In two cell lines, there were cells which contained both GFAP and FN simultaneously. Athymic mouse-borne tumors were developed by the injection of an FN-producing glioma-derived cell line into athymic mice. Human FN plasma levels were monitored, and some tumors larger than 1.0 g produced FN which was detectable in the plasma, thereby demonstrating that tumor-produced FN can be shed from human glioma-derived cells in sufficient amounts to be detectable in the bloodstream. These data show that FN expression in anaplastic human gliomas is seen with the greatest frequency in athymic mouse-borne tumors; GFAP-positive cultured cells are also capable of FN expression. The original tumors rarely express detectable amounts of FN. The biological basis for this heterogeneity of FN expression rests in the nature of the individual tissue studied, its cellular environment (ie, in vivo or in vitro), and its status with regard to neoplasia. (Author abstract) (47 Refs)

43. MODIFICATION OF FIBRONECTIN DISTRIBUTION PATTERN IN SOLID HUMAN TUMOURS.

Labat-Robert J, Birembaut P, Robert L, Adnet JJ
Laboratoire de Biochimie du Tissu Conjonctif (G.R. CNRS No. 40), Fac. Med., Univ. Paris-Val-de-Marne, 94010 Creteil, France
Diagn Histopathol; 4(4):299-306 1981

Fibronectin (FN) distribution (FND) in normal tissue and solid tumors was examined. Indirect immunofluorescence was used to analyze FND in normal breast tissues: FN was found on normal epithelial cell membranes, in periglandular and perivascular basement membranes, and in the intercellular stroma. In infiltrating breast carcinomas, cell membrane staining disappeared even before invasion, and the staining pattern of the glandular basement membranes became discontinuous and disappeared with invasion. Studies on FND in cancers of the bronchi, endometrium, prostate, thyroid, and urinary bladder revealed a loss of typical FND in the tissues. In sarcomas, the FND was considerably altered; loss of pericellular labelling and decreased staining of FN in the extracellular matrix were observed. Immunofluorescence for FND may aid histological identification of atypical lesions and early malignant tumor invasion. (37 Refs)

44. DISTRIBUTION OF FIBRONECTIN ON CLONAL CELL LINES OF A RAT MAMMARY ADENOCARCINOMA GROWING IN VITRO AND IN VIVO AT PRIMARY AND METASTATIC SITES.

Neri A, Ruoslahti E, Nicolson GL
Dept. Biochemistry, Univ. Southern California Comprehensive Cancer Center, Los Angeles, CA, 90033
Cancer Res; 41(12):5082-5095 1981

With the use of a rat 13762 mammary adenocarcinoma tumor, we have examined the relationship between cellular fibronectin (FN) expression and ability to metastasize spontaneously to regional lymphatic and distant blood-borne sites. This model is based on the isolation and establishment of cell clones from primary parental tumor and from spontaneous metastases that show differing metastatic potentials when implanted sc into the mammary fat pads of syngeneic female Fischer 344/CRBL rats. Cellular FN expression was determined in tissue culture as well as at primary and secondary tumor sites, utilizing: (a) indirect immunofluorescence microscopy with a specific anti-rat FN antibody (in vitro and in vivo grown cells); (b) competition radioimmunoassay for cell-released FN (in vitro grown cells); and (c) surface labeling by radioiodination-sodium dodecyl sulfate-polyacrylamide gel electrophoresis-autoradiography for cell surface-bound FN (in vitro grown cells). Tissue culture-grown parental tumor clones displayed FN at their cell surfaces. At confluency, they expressed higher quantities of FN at their peripheries and in fibrillar structures between adjacent cells and released greater amounts of this glycoprotein. Lung metastases-derived tumor clones released negligible amounts of FN by radioimmunoassay and failed to express detectable amounts of FN by indirect immunofluorescence and cell surface-labeling techniques. However, when parental tumor- and metastasis-derived clones of widely different metastatic potentials were carefully examined for FN expression and release, there was no obvious relationship between metastasis and FN expression or release in culture or display in tumors at primary or secondary sites. The results suggest that expression or release of FN per se is not a determinant of metastasis, although it may be a factor in certain steps of the metastatic sequence. (Author abstract) (53 Refs)

45. THE ROLE OF FIBRONECTIN IN ADHESION OF METASTATIC MELANOMA CELLS TO ENDOTHELIAL CELLS AND THEIR BASAL LAMINA.

Nicolson GL, Irimura T, Gonzalez R, Ruoslahti E
Dept. Tumor Biology, Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
Exp Cell Res; 135(2):461-465 1981

Vascular endothelial cells synthesize an extracellular matrix or basal lamina composed of collagens, proteoglycans and glycoproteins such as fibronectin (FN). Using affinity-purified anti-FN, we have examined the role of FN in adherence of metastatic B16 melanoma cells to endothelial cell monolayers which lack FN on apical cell surfaces and to their basal lamina which contains FN. B16 melanoma cells, which do not contain significant amounts of FN, attached at much higher rates to endothelial basal lamina and polyvinyl-immobilized FN compared with intact endothelial cell monolayers. Anti-FN failed to in-

hibit attachment of melanoma sublines of low (B16-F1) or high (B16-F10) metastatic potential to intact endothelial cell monolayers, inhibited slightly B16 cell attachment to basal lamina and completely abolished attachment of B16 cells to polyvinyl-immobilized FN. The antibiotic tunicamycin which inhibits glycosylation of B16 cell surface glycoproteins and blocks experimental metastasis inhibited B16 attachment to endothelial cells, basal lamina and immobilized FN. The results suggest that FN mediates, only in part, the adhesion of B16 melanoma cells to basal lamina through glycoprotein receptors on B16 cells. (Author abstract) (38 Refs)

46. GLYCOLIPIDS: RECEPTORS FOR FIBRONECTIN?

Yamada KM, Kennedy DW, Grotendorst GR, Momoi T
Lab. Molecular Biology, NCI, Bethesda, MD, 20205
J Cell Physiol; 109(2):343-351 1981

Three different assays of fibronectin biological activity were examined for their sensitivity to inhibition by glycolipids. These assays measured cell-cell or cell-substrate adhesive interactions, and quantitate fibronectin's hemagglutinating activity, capacity to mediate cell spreading on plastic dishes, and restoration of normal alignment and morphology to transformed cells. Gangliosides inhibited fibronectin-mediated hemagglutination, cell spreading, and restoration of a normal morphologic phenotype to transformed cells. Of the other lipids tested which were less effective inhibitors, the more negatively charged were the most effective. The most effective ganglioside inhibitors generally contained the most sialic acid residues. The oligosaccharide portions of gangliosides retained inhibitory activity; those with more sialic acid were more effective. These results are consistent with the notion that the 'receptor' for fibronectin on a variety of cell types contains a negatively charged lipid, eg, a ganglioside. Lipids other than gangliosides could conceivably serve as ligands for fibronectin interaction, depending on their concentration on the cell surface. The specificity for certain gangliosides is relative rather than absolute. There is no evidence that fibronectin needs a living cell for its ganglioside-inhibitable activity. Oligosaccharides on glycoproteins may not be fibronectin receptors, since purified glycopeptides did not inhibit hemagglutination. (32 Refs)

47. SIMILARITIES AND DIFFERENCES BETWEEN THE FIBRONECTINS OF NORMAL AND TRANSFORMED HAMSTER CELLS.

Wagner DD, Ivatt R, Destree AT, Hynes RO
(c/o Hynes) E17-227, Massachusetts Inst. Technology, Cambridge, MA, 02139
J Biol Chem; 256(22):11708-11715 1981

Fibronectins from normal and virally transformed hamster cells were compared by several criteria. The fibronectin from transformed cells was similar to that from normal cells in being an intact dimeric glycoprotein with the ability to bind to gelatin, activated thiol-Sepharose, and cells. No evidence was found for proteolytic cleavages or abnormalities in disulfide bonding of transformed cell fibronectin. This fibronectin was also shown to be active in promoting cell attachment, elongation, and alignment. Therefore, the fibronectin produced by transformed cells is not defective. However, it was shown that the transformed cells were partially deficient in their capacity to bind fibronectins from either normal or transformed cells. This deficiency has implications for the significance of the loss of fibronectin on oncogenic transformation. Partial proteolysis of the fibronectins from normal and transformed cells gave rise to the same fragments. However, the glycosylated fragments from transformed cell fibronectin appeared somewhat larger than those from normal cell fibronectin. Analysis of fibronectin glycopeptides showed that transformation leads both to more branches per core and to a higher sialylation of the asparagine-linked complex carbohydrate side chains. (Author abstract) (55 Refs)

OTHER RELATED STUDIES

48. PHENOTYPIC DRIFT OF METASTATIC AND CELL-SURFACE PROPERTIES OF MAMMARY ADENOCARCINOMA CELL CLONES DURING GROWTH IN VITRO.

Neri A, Nicolson GL
(c/o Nicolson) Dept. Tumor Biology, Univ. Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
Int J Cancer; 28(6):731-738 1981

Cell clones obtained from a 13762 mammary adenocarcinoma tumor and its spontaneous metastasis for phenotypic stability during serial culture passage in vitro were examined. Two clones were chosen for further examination. One of these clones (MTC) obtained from the parental transplanted tumor initially failed to metastasize within 23 days post-injection sc but gained the ability to form spontaneous pulmonary metastases after several serial passages in vitro. Another clone (MTLn3) derived from a spo ntaneous lung metastasis was initially highly metastatic after short-term culture, but lost the potential to form large numbers of spontaneous lung metastases with long-term culture. In contrast to MTC, clone MTLn3 displayed lymph-node metastasis, and the frequency of lymph-node involvement increased when late-passage cultures of MTLn3 cells were assayed in vivo. Both clones from late-passage cultures produced larger tumor sizes at the primary injection sites compared to early passage cells. The morphologies of MTC cells changed with serial tissue culture passage, while the morphologies of MTLn3 cells did not change. The display of fibronectin on MTC cells by immunofluorescence did not change with culture passage; fibronectin was not detected in cultures of clone MTLn3. Fibronectin was also found on MTC cells by cell surface labelling using lactoperoxidase-catalyzed iodination-sodium dodecylsulfate polyacrylamide gel electrophoresis-autoradiography. Iodination of fibronectin on MTC cells did not vary with culture passage, and as in immunofluorescence experiments it was not detected on MTLn3 cells. There was a decrease in exposure of certain cell surface proteins on MTC cells with culture passage, but we did not detect modifications with this procedure, that correlated with culture passage of MTLn3 cells. We conclude that prolonged culture in vitro can result in modifications of metastastic and cell-surface properties of tumor cell clones. (51 Refs)

49. CHANGES OF THE INTERNAL ORGANIZATION OF THE PLASMA MEMBRANE CORRELATED TO THE REGENERATION POTENCY OF THE CELL.

Speth V, Bauer HC, Brunner G
Max-Planck-Institut fur Immunobiologie, 7800 Freiburg, W. Germany
Biochim Biophys Acta; 649(1):113-120 1981

Internal organization of the plasma-membrane of rat thymocytes and pituitary tumor cells (GH3) was experimentally altered by low temperature and either changing osmolarity or adding drugs destroying the cytoskeleton. These treatments

induce reversible aggregation of intramembrane particles of the plasma membrane. Thin section electron microscopy of the hypotonically shocked cells show all the cell organelles to be extremely swollen and the cytoplasmic space to be rather empty. Returned to physiological conditions, the cells show normal morphological aspects, accompanied by 'normal' permeability properties of the plasma membrane; the aggregation of intramembrane particles is reversed. The proliferation behavior of GH3-cells is not affected by this treatment. This demonstrates a high regeneration potency of the mammalian cell and leads to the assumption that molecular components which are important for the survival of the cell must be structural (membrane) bound. (Author abstract) (13 Refs)

50. PLASMA MEMBRANE ALTERATIONS DUE TO PROMOTERS AND ANTIPROMOTERS.

Braun AG, Buckner CA, Nichinson BB
Dept. Radiation Therapy, Harvard Medical Sch., 50 Binney St., Boston, MA
Teratogenesis Carcinog Mutagen; 1(4):417-427 1981

The effect of various agents on the attachment of (Mouse Ovarian Tumor) cell to plastic surfaces coated with Concanavalin A (Con A) was investigated. Promoters and the majority of antipromoters tested were found to inhibit attachment. All tested nonpromoters except for Aroclor 1254 did not affect attachment. Promoters could be distinguished from antipromoters by their effect on the apparent microviscosity of tumor cell plasma membranes. At inhibitory concentrations, promoters reduced microviscosity while antipromoters increased microviscosity. Microviscosity changes due to drug treatment were detected by fluorescent polarization measurements of an intercalated membrane probe, diphenylhexatriene. Based on these results, a simple in vitro screen for the detection of promoting agents in the environment may be possible. (42 Refs)

51. EVIDENCE OF FAR ULTRAVIOLET LIGHT-MEDIATED CHANGES IN PLASMA MEMBRANE STRUCTURE AND FUNCTION.

Braun AG, Buckner CA
Dept. Radiation Therapy, Harvard Medical Sch., 50 Binney Street, Boston, MA, 02115
Biochim Biophys Acta; 648(2):263-266 1981

Irradiation of plasma membrane preparations with 254 nm light increases its apparent microviscosity as measured with fluorescent polarimetry. Doses of 3.10(4) J/m2 increase the fluorescent polarization of a diphenylhexatriene probe by 10%. A similar increase is seen when whole cells are irradiated. The fate of membrane protein following irradiation was examined using SDS-polyacrylamide gel electrophoresis. Increasing the 254 nm doses reduces the amount of material in distinct bands on the gel and increases the amount of very low mobility material. No new bands of Coomassie blue staining material were observed. Irradiation of whole cells inhibited their attachment to concanavalin A-coated surfaces, an indication of a change in membrane function. (Author abstract) (12 Refs)

52. THE EFFECT OF OXIDANT INJURY ON THE LYMPHOCYTE MEMBRANE AND FUNCTIONS.

Kraut EH, Sagone AL
Ohio State Univ. Hosp. N 1025, 410 West 10th Ave., Columbus, OH, 43210
J Lab Clin Med; 98(5):697-703 1981

We have studied the effect of oxidant stress on the lymphocyte membrane and lymphocyte functions. Lymphocyte cultures were incubated with xanthine oxidase and xanthine, an enzyme system known to generate several highly reactive oxygen compounds. We demonstrated that these lymphocytes were viable after exposure to an in vitro oxidant stress. However, there was a marked reduction in their ability to bind sheep RBCs and to form caps after Concanavalin A stimulation. These lymphocytes also demonstrated a delay in phytohemagglutinin-induced lymphoblastic transformation, with max response occuring at 5 days instead of 3 days. Catalase, a hydrogen peroxide scavenger, protected lymphocytes from this injury, implicating hydrogen peroxide as the causative agent. Another lymphocyte membrane-related function, the ability to stimulate or respond in mixed leukocyte culture, was not impaired after oxidant injury. These results demonstrate that after in vitro oxidant injury, lymphocytes may have alterations in the cell membrane and impaired function. (Author abstract) (24 Refs)

53. MEMBRANE FLUIDITY AND CELL-SURFACE RECEPTOR MOBILITY (ONCOLOGY OVERVIEW).

International Cancer Research Data Bank
NCI, Westwood Building, Room 10A18, Bethesda, MD
Membrane Fluidity and Cell-Surface Receptor Mobility (Oncology Overview) Cooper, R, ed This Title is Available Through National Technical Information Service, Springfield, VA, as NTISUB/PB81-923004:; OB-81/04:52pp. 1981

ONCOLOGY OVERVIEW is a service of the International Cancer Research Data Bank (ICRDB) Program of the National Cancer Institute. Retrospective bibliographies containing 100-500 selected abstracts of published research on topics of high interest to cancer investigators are presented. This OVERVIEW emphasizes the microviscosity and surface receptor mobility of neoplastic and normal cell membranes. Specific emphasis is given to studies dealing with the methodology of specific biophysical membrane probes. Also of interest are papers which examine the contribution of different membrane components to microviscosity, as well as the effect of drugs and various treatments on membrane fluidity. The section of papers concerning cell-surface receptor mobility emphasizes lectin receptors and receptors involved in the immune response.

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. GANGLIOSIDE PROFILES OF METASTASES AND OF METASTASIZING AND NONMETASTASIZING RAT PRIMARY MAMMARY CARCINOMAS.

Skipski VP, Carter SP, Terebus-Kekish OI, Podlaski FJ, Peterson RH, Stock CC
Donald S. Walker Lab., Sloan-Kettering Inst. Cancer Res., 145 Boston Post Road, Rye, NY, 10580
JNCI; 67(6):1251-1258 1981

The possible relationship between ganglioside levels and ganglioside profiles in malignant tumors and the formation of metastasis was investigated by the analysis of gangliosides in metastasizing SMT-2A and nonmetastasizing MT-W9a mammary carcinomas as well as in metastases formed from SMT-2A tumors. The extracted lipid of SMT-2A tumors contained 3.3-

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fold more lipid-bound sialic acid than did that of MT-W9a tumors. The differences were also substantial in the ganglioside profiles in these two tumors. Plasma membranes isolated from SMT-2A tumors also contained 1.8-fold more lipid-bound sialic acid than did plasma membranes from MT-W9a tumors. Ganglioside profiles in two types of SMT-2a secondary tumors were investigated. The lipid-bound sialic acid content was 1.5-fold higher in tumor nodules in the lung and 1.9-fold higher in axillary lymph node tumors than it was in primary SMT-2A tumors. The ganglioside pattern in these two secondary tumors generally reflected that found in SMT-2A: high levels of gangliosides containing three or four sialic acid molecules. The lung nodule retained its specificity with respect to lipid-bound sialic acid content and ganglioside pattern after the lung nodule was sequentially transplanted three times to the site of the original SMT-2A tumor growth. (Author abstract) (46 Refs)

2. RAPID ISOLATION AND LIPID CHARACTERIZATION OF PLASMA MEMBRANES FROM NORMAL AND MALIGNANT LYMPHOID CELLS OF MOUSE.

Koizumi K, Shimizu S, Koizumi KT, Nishida K, Sato C, Ota K, Yamanaka N
Lab. Chemotherapy, Aichi Cancer Center Res. Inst., Nagoya 464, Japan
Biochim Biophys Acta; 649(2):393-403 1981

A rapid isolation method was developed for plasma membranes from mouse lymphoid cells such as lymph node lymphocytes, thymocytes, radiation-induced thymoma cells and L1210 cells. Lysates of these lymphoid cells were prepared by Dounce homogenization under hypotonic conditions and directly layered on sucrose step density gradients containing 2 mM CaCl2 and 5 mM MgCl2, and centrifuged at 52,000 x g for 1 hr. Plasma membrane fractions appeared at the interface between 20 and 42% sucrose in the gradients. The procedure permitted purified membranes from cells to be obtained within 3 hr, and the preparations, appeared to be uniform by electron microscopy. Specific activities of (Na+ + K+)-ATPase, Mg++-ATPase and 5'-nucleotidase of the isolated plasma membranes were enriched 23-to 61-fold, 12-to 15-fold and 18-to 34-fold, respectively, in comparison with those of the corresponding cell homogenates. Cholesterol content of the malignant cell membranes was lower than that of the normal membranes and the molar ratio of cholesterol to phospholipid of the malignant cell membranes was also lower than that of the normal membranes. A decreased plasmalogen content was observed in the malignant plasma membranes, together with a higher percentage of phosphatidylethanolamine and a lower percentage of phosphatidylserine. In the normal cell membranes, thymocytes contained a higher percentage of phosphatidylcholine and a lower percentage of sphingomyelin than those of the lymph node lymphocytes. At all temperature ranges (5 to 40 C) the plasma membranes of the malignant cells had lower microviscosity than those of the normal cells. (Author abstract) (43 Refs)

3. PREPARATION AND BIOLOGICAL ACTIVITY OF FATTY ACID DIMER.

Balu CR, Ramaiah TR
Dept. Postgraduate Studies and Res. in Biochemistry, Univ. Mysore, Manasagangotri, Mysore 570 006, India
Indian J Biochem Biophys; 18(2):124-127 1981

Ricinoleic acid was dimerized by dehydrating the secondary alcohol group with either Fuller's earth or concentrated sulfuric acid. Cytopathic effects of castor oil hydrolysate (CH) were then compared with those of fractions of sulfuric acid dimerized samples from a silica gel column. No cytopathic effect occurred in HeLa cells treated for 2.5 hr with CH. In contrast, incubation with either the ether or alcohol fractions prepared with concentrated sulfuric acid resulted in a pronounced cytopathic effect within 15 min. After a brief period of incubation, small extrusions of the cell membrane appeared, followed eventually by membrane rupture and cell death. The effect of these samples may thus be at the membrane level. Since only the more polar fractions of the sample were cytopathic, the polarity of the molecules may have some role in this effect. (13 Refs)

4. NEUTRAL GLYCOSPHINGOLIPIDS OF RAT LIVER AND HEPATOMAS: COMPOSITION, BIOSYNTHESIS AND ALTERATIONS IN TUMORIGENESIS.

Walter VP
Purdue University, Lafayette, IN, 47907
Diss Abstr Int [B]; 42(5):1725-B 1981

Neutral glycosphingolipids (NGSL) undergo alterations as one result of the tumorigenic process. The purpose of this study was to examine these alterations in transplanted hepatoma lines and to determine if selective enzymatic synthesis was responsible. NGSL were extracted and purified from transplanted hepatomas, from sera of tumor-bearing rats, and from livers of rats fed the carcinogen 2-acetylaminofluorene and compared to livers and sera of control animals. To quantitate NGSL at the cell surface, a method of plasma membrane isolation from transplanted hepatomas was adapted. Lastly, the biosynthesis of ceramide, glucosylceramide, and lactosylceramide were examined in these same tissues. (no Refs)

5. PROTEIN GLYCOSYLATION IS REQUIRED FOR THE PROLIFERATION OF MITOGEN-ACTIVATED LYMPHOCYTES.

Harrison ML, Villemez CL
Tumor Immunology, Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
J Immunol; 127(6):2616-2617 1981

The biochemical events underlying the lymphocyte activation process are at present not understood, although it is clear that surface components play a crucial role. In vitro, activation can be induced by specific mitogens such as bacterial lipopolysaccharide (LPS; specific for B lymphocytes) or the plant lectin concanavalin A (Con A, specific for T lymphocytes). One result of lymphocyte mitogenesis is a dramatic increase in cellular proliferation. It is clear that the activation signal provided by the mitogen results from an initial contact between the mitogen and the lymphocyte surface, presumably through a specific membrane receptor. Other membrane components that may play an essential role in inducing or maintaining the proliferative state have not been identified. Through work involving the glycosylation of lymphocyte proteins, we observed that inhibition of protein glycosylations occurring via the lipid intermediate (dolichol) pathway resulted in the prevention of lymphocyte activation. The antibiotic tunicamycin (TM)(3) specifically inhibits glycosylations occurring via the lipid intermediate pathway. We report here that nanomolar quantities of TM prevented lymphocyte proliferation induced by either Con A or LPS. In direct contrast, TM had no effect on the proliferation of nonactivated lymphocytes, BW147.3 lymphatic leukemic cells, H4-II-E-C3 hepatoma cells, or Escherichia coli B bacteria. (Author abstract) (24 Refs)

6. CLONING OF A FUNCTIONAL GENE RESPONSIBLE FOR THE EXPRESSION OF A CELL SURFACE ANTIGEN CORRELATED WITH HUMAN CHRONIC LYMPHOCYTIC LEUKEMIA.

Stanners CP, Lam T, Chamberlain JW, Stewart SS, Price GB
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Cell; 27(1,part2):211-221 1981

A cell-cell hybrid of a Chinese hamster ovary (CHO) cell line and human peripheral blood lymphoblasts from a patient with B-cell chronic lymphocytic leukemia produces a surface antigen detectable by a monoclonal antibody. This surface antigen can be detected at significant levels on a fraction of chronic lymphocytic leukemia cells, but not on normal human lymphocytes from peripheral blood or bone marrow. Two different clones capable of transforming mouse cells to produce the surface antigen were isolated from a gene library of the hybrid cell by identification of small portions of the library containing functional genes, followed by detection of clones with human-specific reiterated sequences. Both clones also contain CHO-specific reiterated sequences and are therefore human-CHO recombinants. From the frequency of antigen-producing clones in the library, we estimate that they were present in the hybrid cell genome at a copy number of 100 to 1,000. (Author abstract) (32 Refs)

7. WANDERING AROUND THE CELL SURFACE-MONOCLONAL ANTIBODIES AGAINST HUMAN NEUROBLASTOMA AND LEUKEMIA CELL SURFACE ANTIGENS (MEETING ABSTRACT).

Feder M, Jonak Z, Smith A, Glick MC, Kennett RH
Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104
Hybridomas and Cellular Immortality (Second Houston Symposium), November 5-6, 1981, Houston, Texas. University of Texas Medical School at Houston 55 pp., 1981.

Monoclonal antibodies have been made against cell surface antigens on human neuroblastoma cell lines and leukemia cell lines. These antibodies were tested against a panel of human cell types to define those antibodies having a restricted specificity. We will describe the use of monoclonal antibodies for detection of metastasis of neuroblastoma cells to bone marrow. Using a combination of two monoclonal antibodies we are able to detect and identify neuroblastoma cells and to distinguish them from normal and malignant lymphoid cells. We have also used these antibodies to isolate the cell surface glycoproteins by affinity chromatography. The glycoprotein antigens isolated from different tumor cell lines have been isolated and compared. One of the most promising applications of anti-tumor monoclonal antibodies may be the detection of cell surface molecules involved in the processes of cell growth and cell division. We have identified three monoclonal antibodies which inhibit the growth of the leukemia cell line Reh. The detection of these antibodies and their use in characterization of cell surface receptors will be discussed. (no Refs)

8. A BIOCHEMICAL AND BIOSYNTHETIC ANALYSIS OF HUMAN-MELANOMA-ASSOCIATED ANTIGENS WITH MONOCLONAL ANTIBODIES (MEETING ABSTRACT).

Bumol TF, Harper JR, Chee DO, Reisfeld RA
Scripps Clinic and Research Foundation, La Jolla, CA, 92037
Hybridomas and Cellular Immortality (Second Houston Symposium), November 5-6, 1981, Houston, Texas. University of Texas Medical School at Houston 55 pp., 1981.

Two monoclonal antibodies (MoAbs 9.2.27 and F11) directed to cell surface and secreted melanoma associated antigens, respectively, were used to study the expression, biosynthesis and molecular nature of these glycoconjugated molecules. MoAb 9.2.27 detected from 1-2 x 10(5) antigenic sites per cell in direct binding studies of several melanoma cell lines. Analysis of subcellular fractions of these cells by the same method indicated that nearly all antigenic activity was in crude membrane fractions. Sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) analyses of immunoprecipitates of detergent extracts from biosynthetically labelled M21 melanoma cells showed that MoAb 9.2.27 recognizes a 250 kilodalton (250K) molecule together with a high mol wt component (HMW-C), Mr greater than 500,000 dalton. Pulse-chase analysis of this antigen complex in extracts of 35S-methionine labelled cells, treated with either the monovalent ionophore monensin or endoglycosidase H, revealed that MoAb 9.2.27 recognizes the 250K N-linked glycoprotein in the absence of the HMW-C as well as several precursor glycoproteins ranging from 210-240K. The chondroitin sulfate nature of the HMW-C was indicated by its ability to label with 35S-sulfate ion and its sensitivity to beta elimination and proteoglycan-specific enzymes chondroitinase ABC and AC lyases. MoAb F11 recognizes a glycoprotein of 100 kilodalton (100K) that has limited cell surface expression a s it is secreted into spent culture media of melanoma cells. Pulse-chase analysis revealed the 100K antigen to be secreted at a slower rate than other major melanoma-associated glycoproteins, ie, fibronectin, suggesting a unique biosynthetic pathway. The 100K antigen is heavily glycosylated as evidenced by sensitivity to neuraminidase and biosynthetic alterations induced by monensin. Two monoclonal antibodies could thus be used effectively for an in depth study of biosynthetic and biochemical aspects of glyco-conjugated tumor associated antigens, paving the way for an analysis of their biological function in metastatic melanoma. (no Refs)

9. MONOCLONAL ANTIBODIES TO ANTIGENS OF HUMAN MELANOMA (MEETING ABSTRACT).

Brown JP, Hellstrom I, Hellstrom KE
No affiliation given
Hybridomas and Cellular Immortality (Second Houston Symposium), November 5-6, 1981, Houston, Texas. University of Texas Medical School at Houston 55 pp., 1981.

Monoclonal antibodies were used to identify and characterize antigens of human melanoma. Spleen cells from mice immunized with melanoma cells were fused with mouse myeloma cells. The hybrids were screened both by binding assays and by immunoprecipitation. One of the most interesting antigens identified is p97, a 97,000 mol wt cell surface glycoprotein, which is present in most melanomas but in only trace amounts in normal adult tissue. However, certain fetal tissues contain significant amounts of p97. Monoclonal antibodies to a number of distinct antigenic determinants of p97 have been used to develop a 2-site immunoradiometric assay, to obtain syngergistic complement-dependent cytotoxicity, for preparation of immunotoxins and for imaging studies. P97 has been purified from detergent-lysates of cultured melanoma cells by affinity chromatography with monoclonal antibody and Staphylococcus aureus protein A coupled to Sepharose. Amino acid sequencing studies of the purified protein have revealed that the N-terminal amino acid sequences of p97 and transferrin are homologous. The structure homology of p97 and transferrin was confirmed serologically using a mouse antiserum to denatured p97. Like transferrin, p97 binds iron. We conclude that melanoma-associated antigen p97 is evolutionarily and functionally related to transferrin, although its exact role in melanoma cells remain to be elucidated. (no Refs)

10. PHOSPHOLIPASE ACTIVITY IN SKIN AFTER APPLICATION OF PHORBOL ESTERS AND 3-METHYLCHOLANTHRENE.

Bresnick E, Bailey G, Bonney RJ, Wightman P

Dept. Biochemistry, Vermont Regional Cancer Center, Lake Champlain Cancer Res. Assoc., Univ. Vermont Coll. Medicine, Burlington, VT, 05405

Carcinogenesis; 2(11):1119-1122 1981

Topical administration of the promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), is accompanied by an increased incorporation of [3H]arachidonic acid into mouse skin and a very signi ficant activation of epidermal cell membrane phospholipase A2 without affecting intracellular acid phospholipase. Similar enhancement in epidermal cell phospholipase A2 was observed after application of phorbol-12,13-didecanoate or 3-methyl-cholanthrene to mouse skin. A small but significant increase in phospholipase A2 activity is also seen after application of the nonpromoter irritant, acetic acid. The elevated levels of the prostaglandins observed in mouse skin after topical application of promoters are probably triggered by the activation of this membrane enzyme. (Author abstract) (25 Refs)

11. INTRACELLULAR ALKALINE PHOSPHATASE ACTIVITY IN CULTURED HUMAN CANCER CELLS.

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Histochemistry; 73(1):1-13 1981

The validity of saponin (SP) treatment in demonstrating intracellular alkaline phosphatase (AP) activity was studied in the human cancer cell lines KMK-2, HeLa S3-10, and BeWo. AP cytoplasmic activity was demonstrable after SP treatment. Measurement of cell 'pellet' AP indicated a significant increase in specific activity due to SP. Bromelain was used to cleave AP in the living cell membrane to remove any source of diffusion artifact; intracellular membrane sites were evident in such cell preparations. The extent of increase in AP due to SP was more marked in bromelain-treated than untreated HeLa S3-10 cells. AP released from the cells in this way was itself not affected by SP treatment, suggesting that SP may act by altering the membrane rather than the enzyme itself. BeWo cells exhibited sodium butyrate-induced AP in the cytoplasm. KMK-2 cells exhibited positive reactions for AP in the perimitochondrial membrane, in cisternae of the endoplasmic reticulum, and in the Golgi apparatus. Intracellular or even plasma membrane AP demonstrated after SP treatment appeared more resistant to the inhibitory effect of levamisole than that of the plasma membrane which is demonstrable without SP. The intracellular reaction apparently did not result from artifact. Few HeLa S3-10 cells showed intracellular AP activity. The results indicated that SP treatment is useful in demonstrating intracellular and even cryptic plasma membrane AP activity. (22 Refs)

MORPHOLOGICAL STUDIES

12. ULTRASTRUCTURAL BASAL LAMINA ALTERATIONS AT THE TUMOR-STROMA JUNCTION IN INVASIVE LARYNGEAL CARCINOMA.

Schenk P

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Laryngol Rhinol Otol (Stuttg); 60(11):573-576 1981

Normal and pathological samples of laryngeal epithelium from 16 patients with histologically confirmed invasive squamous cell carcinoma of the larynx, were studied by light- and electron-microscopy. Normal tissue exhibited a continuous, 40-nanometer-wide basal lamina (BL) associated with the dermal surface of the plasma membrane of basal cells at the junction with the stroma. The BL was a product of the epithelial cells and had numerous cytoplasmic projections and dermal invaginations. In the tumor-stroma junction the BL was thinner than normal, discontinuous, or completely absent from extended areas of the junction. Abnormal occurrence of BL between lateral surfaces of adjacent malignant basal keratinocytes. These abnormal BL showed multiple, deep invaginations and projections with lumina of various sizes. The cellular membrane associated with the abnormal BL was marked by numerous hemidesmosomes. Anchor filaments were observed between BL and microvilli in the intercellular spaces. A possible barrier function of BL in tumor invasion was indicated. Disruption of the BL was not regarded as a criterion for tumor invasion in laryngeal carcinoma. (14 Refs) (In GER)

13. ULTRASTRUCTURAL STUDY ON THE CELL SURFACE OF HUMAN E-ROSETTING AND LEUKEMIC LYMPHOBLASTS.

Koscielniak E, Sporniak M, Chybicka A

Pediatric Clinic, Acad. Medicine, Warszawa, Poland

Folia Histo[illegible]a Cytochem (Krakow); 19(3):34-35 1981

Alterations in cell surface morphology were compared in lymphocytes and in acute lymphoblastic leukemia lymphoblasts forming E-rosettes with receptors for sheep erythrocytes (SRBC). Two types of junction between SRBC and lymphoid cells were found. Results point to some functional differences at the level of the plasma membrane between normal and leukemic lymphocytes in the formation of nonimmune rosettes. (no Refs)

MEMBRANE RECEPTOR/ BINDING SITES

14. HIGH AFFINITY BINDING OF 125I-LABELED MOUSE INTERFERON TO A SPECIFIC CELL SURFACE RECEPTOR. II. ANALYSIS OF BINDING PROPERTIES.

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Virology; 115(2):249-261 1981

Direct ligand-binding studies with highly purified 125I-labeled virus-induced mouse interferon on mouse lymphoma L1210 cells revealed a direct correlation of specific high-affinity binding with the biologic response to interferon. Neutralization of the antiviral effect by anti-interferon gamma globulin occurred at the same antibody concentration as the inhibition of specific binding. These results suggest that specific high-af-

finity binding of 125I-interferon occurred at a biologically functional interferon receptor. Competitive inhibition experiments using 125I- and 127I-labeled interferon provided strong evidence that the fraction of 125I-interferon inactivated upon labeling did not bind specifically. Scatchard analysis of the binding data yielded linear plots and thus suggested that interferon binds to homogeneous noncooperative receptor sites. In contrast to a characteristic property of several peptide horm one systems, binding of 125I-interferon to its specific receptor did not induce subsequent ligand degradation. At 37 C bound interferon was rapidly released in a biologically active form without evidence for molecular degradation. The expression of interferon receptors was not modified by treatment with interferon. Trypsin treatment of target cells and inhibition of protein synthesis abolished the specific binding of 125I-interferon. Three major mol wt species of Newcastle disease virus-induced mouse C 243 cell interferon were isolated separately and identified as mouse alpha and beta interferons. These interferons were shown to inhibit competitively the specific binding of the highly purified labeled starting material which contained all three size species, thus providing evidence for a common receptor site for mouse alpha and beta interferon. (Author abstract) (24 Refs)

15. DERMORPHINS, OPIOID PEPTIDES FROM AMPHIBIAN SKIN, ACT ON OPIOID RECEPTORS OF MOUSE NEUROBLASTOMA X RAT GLIOMA HYBRID CELLS.

Glaser T, Hubner K, De Castiglione R, Hamprecht B
(c/o Hamprecht), Physiologisch-Chemisches Institut der Universitat, Koellikerstr, 2, 8700 Wurzburg, W. Germany
J Neurochem; 37(6):1613-1617 1981

Dermorphin and its Hyp6 analogue are opiate-like heptapeptides originally discovered in frog skin and characterized by the presence of a D-Ala2 residue in their sequence. They were assayed for their capacity to compete with [3H]Leu-enkephalin for binding to opioid receptors in membranes of neuroblastoma x glioma hybrid cells. In the presence of 7 nM-[3H]Leu-enkephalin, the concentrations at which they caused 50% inhibition of [3h]enkephalin binding (IC50 values) are 0.1 uM and 0.3 uM, respectively. In contrast, the synthetic L-Ala(2)-dermorphin shows very low affinity for the opioid receptors. In addition, like other opioid peptides, dermorphin and Hyp(6)-dermorphin inhibit the elevation by prostaglandin E1 (PGE1) of the level of cyclic AMP (IC50 values 0.2 uM and 0.4 uM, respectively). The inhibition is prevented by the opiate antagonist naloxone. L-Ala(2)-dermorphin is at least three orders of magnitude less potent in inhibiting the PGE1-evoked increase in the level of cyclic AMP. The results show that peptides with an amino acid sequence quite different from that of the enkephalins can bind to opioid receptors of the hybrid cells. (Author abstract) (29 Refs)

16. AFFINITY CYTOCHEMISTRY VISUALIZES SPECIFIC ESTROGEN BINDING SITES ON THE PLASMA MEMBRANE OF BREAST CANCER CELLS.

Nenci I, Marchetti E, Marzola A, Fabris G
Istituto di Anatomia e Istologia Patologica, Univ. Ferrara, 44100 Ferrara, Italy
J Steroid Biochem; 14(11):1139-1146 1981

A cytochemical technique involving estradiol labeled covalently with macromolecular fluorescent probes was used to detect possible estrogen-binding sites on the plasma membrane of viable human breast cancer cells. At 4 C, breast cancer cells, but not other non-epithelial cell types, showed bright fluorescence diffusely distributed over the cell surface and in a ring pattern. Binding occurred by a saturable pathway, because pretreatment of cells with the same ligand in 'cold' form abolished labeling. Non-estrogen steroid hormones, synthetic estrogen, and anti-estrogen did not hinder binding, indicating that binding is structurally specific for natural estrogen. Binding sites showed temperature-sensitive lateral mobility in the plane of the membrane, which led to formation of small clusters, patches, and polar caps at 23 or 37 C. Results indicate that estrogen-target cells have plasmalemmal estrogen-binding sites, which do not involve classical cytoplasmic receptors. Translational mobility of binding sites is consistent with their potential receptor nature. (40 Refs)

TRANSPORT STUDIES

17. STIMULATION OF NA INFLUX, NA-K PUMP ACTIVITY AND DNA SYNTHESIS IN QUIESCENT CULTURED CELLS.

Rozengurt E
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Adv Enzyme Regul; 19:61-85 1981

A central problem in cell regulation is to elucidate the biochemical basis of the metabolic activation seen in quiescent cells stimulated to proliferate. Recent evidence has shown that an increase in the rate of Na influx is one of the earliest events in the stimulation of quiescent cells into DNA synthesis and cell division. This phenomenon has been demonstrated in a variety of fibroblastic cells and it is elicited by serum, as well as by purified growth factors. The stimulation of Na influx is apparently sufficient to increase the activity of the Na-K pump of the plasma membrane. The activity of this enzyme is sensitive to small fluctuations of cellular Na, as shown by using the selective Na ionophore monensin, which increases cellular Na and Na-K pump activity. A number of recent findings suggest that the influx of Na ions into the cell can signal later events of cell proliferation, specifically the initiation of DNA synthesis. The evidence supporting this notion is as follows: (a) the rate of Na entry rapidly increases in a variety of quiescent fibroblastic cells stimulated to proliferate by serum or purified growth factors; (b) a decrease in Na entry by lowering the Na concentration in the extracellular medium or by inhibiting the uptake of Na by amiloride markedly blocks the initiation of DNA synthesis promoted by serum or growth factors; (c) the neurohypophyseal nonpeptide vasopressin, the potent tumor promoter TPA, the amphipathic polypeptide melitin and the channel-forming antibiotic amphotericin B stimulate Na ion influx and trigger the initiation of DNA synthesis, acting synergistically with other growth factors; (d) certain cells transformed by oncogenic viruses show an increased Na flux as compared with their normal counterparts. These findings suggest that Na influx may signal the initiation of mitogenesis in quiescent cells. (Author abstract) (84 Refs)

18. CHANGES IN THE TRANSPORT OF 14C-LEUCINE ACROSS THE ERYTHROCYTE MEMBRANE IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA.

Stepniewski M, Armata J, Cyklis R, Nawrocka-Kanska B, Szafran Z
Zaklad Biochemii Klinicznej, ul. Wielicka 265, 30-663 Krakow, Poland
Przegl Lek; 38(7):547-549 1981

The transport of 14C-leucine across the RBC membrane was studied in 19 children (6 boys and 13 girls, 2-14 yr old, mean age 5.5 hr) with acute lymphoblastic leukemia. The test was carried out immediately after diagnosis, ie, before treatment, in 13 cases (Group I), and during remission achieved by chemotherapy in 6 cases (Group II). Twenty-two nonleukemic children (2-11 yr-old) served as controls. The coefficient of leucine distribution between the intracellular water of the RBC and the incubation medium was highest in Group I (0.97 +-0.03 versus 0.72 +-0.03 in the controls p less than 0.001). The distribution coefficient was significant lower in Group II (0.79 +-0.04) than in Group I, but did not differ significantly from that in the controls. The findings indicate the existence of structural changes in leukemic RBC membranes, which are responsible for the observed changes in leucine transport. (12 Refs) (In POL)

19. HIGHLY-PERMEABLE INTERCELLULAR COMMUNICATIONS AND ELECTRICAL CHARACTERISTICS OF NORMAL LIVER AND HEPATOMA. I. HIGHLY-PERMEABLE INTERCELLULAR COMMUNICATIONS IN INDUCED MURINE HEPATOMA.

Sharovskaia IuIu, Mittel'man LA, Smolianinov VV, Chailakhian LM
Res. Lab. Molecular Biology and Bio-organic Chemistry, M. V. Lomonosov State Univ., Moscow, USSR
Tsitologiia; 23(10):1142-1148 1981

Permeability of the intercellular communications was studied in C3HA mice with o-aminoazotoluene-induced hepatomas. Transmembrane potentials, the input resistance, and distribution of the electric potential measured in hepatoma tissue at different stages of carcinogenesis did not differ from those in normal liver tissue. It was suggested that highly-permeable intercellular communications play a nonspecific role in malignant transformation. (12 Refs) (In RUS)

MEMBRANE BIOGENESIS AND METABOLISM

20. THE DYNAMIC STATE OF LIVER GAP JUNCTIONS.

Yancey SB, Nicholson BJ, Revel JP
Div. Biology, California Inst. Technology, Pasadena, CA, 91125
J Supramol Struct; 16(3):221-232 1981

Incorporation of the radiolabeled amino acid precursors 3H-leucine and 35S-methionine (SM) into the gap junction protein was monitored by using a rapid method for the isolation of gap junctions (GJ) from small amounts of rat liver (2-3 g). Studies with SM as a precursor showed that the specific activity in the protein reaches max 4 hr after a single injection of 300 uCi/100 g body wt. GJ protein has an apparent half-life (HL) of about 19 hr judging from the decay in the specific activity with time after a single injection. Apparent HL probably overestimates the true HL because of the problems with the reutilization of radiolabeled amino acid with SM as precursor. Surprisingly rapid turnover of GJ protein is indicated. Short HL suggests that in rat liver the GJ may be very responsive to alterations in physiological demands. (40 Refs)

21. MEMBRANE EXPRESSION OF FC-RECEPTORS IN CULTURED LEUKEMIC CELL LINES I: INDUCTION OF FC-RECEPTOR IN UNDIFFERENTIATED TYPES OF CELLS AFTER PASSIVE MODULATION OF LIPID VISCOSITY.

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J Immunol; 127(6):2252-2256 1981

The role of lipid environment of plasma membrane for the expression of Fc receptor (gamma) (FcRgamma) were studied by physicochemical modification of the membranes of myeloid leukemia cell line cells, M1. Sterol binding polyene antibiotics such as amphotericine B or filipin inhibit the expression of FcRgamma during the differentiation of M1-cells by the CM obtained from the culture of mouse embryonic fibroblasts, suggesting the possible involvement of cholesterol molecules in the induction mechanism of FcRgamma. Low-temperature incubation at either 20 C or 4 C results in the increment of their membrane microviscosity, when tested by the fluorescence depolarization method, in company with the appearance of FcRgamma on approximately 60% of M1 cells when assayed by the EA-rosetting method. FcRgamma is also induced on approximately 40% of M1-cells by incubating cells with cholesterol-phosphatidylcholine liposomes with the concomitant increase of their membrane microviscosity. Nevertheless, no phagocytosis of EA is observed in these cells treated with such physicochemical procedures. These findings imply that FcRgamma is embedded in the membranes of M1-cells and that physicochemical modification of the membrane lipid bilayers can induce the FcRgamma independent of their functional differentiation marked by the expression of phagocytic activity. (Author abstract) (27 Refs)

22. EFFECTS OF TUNICAMYCIN ON ANTHRACYCLINE RESISTANCE IN P388 MURINE LEUKEMIA CELLS.

Chou TH, Kessel D
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Biochem Pharmacol; 30(22):3134-3136 1981

The inhibition of glycoprotein synthesis in an adriamycin (ADR)-resistant subline of P388 murine leukemia (P388/ADR) was studied for its effects on anthracycline responsiveness and drug efflux. Labeled daunorubicin (DR), cross resistant with P388/ADR in vivo, was used. Cell surface effects of t unicamycin (TC) were independently monitored by a two-phase partitioning method. TC inhibited fucose incorporation indicative of glycoprotein synthesis by 60%. TC increased the partition coefficient of both P388 and P388/ADR cells before and after neuraminidase treatment. Partitioning behavior was primarily affected by degree of membrane sialation, not by TC binding to cell surface. TC did not affect the capacity of P388 and P388/ADR cells for concentrative uptake of cycloleucine, a non-metabolized amino acid. P388 and P388/ADR viability was decreased by approx 50% following 1 hr exposure to 0.04 ug/ml and 0.060 ug/ml DR, respectively. TC (3 ug/ml) inhibited P388 and P388/ADR cell growth for 24 hr; after that time normal growth resumed. Exposure of TC-treated cells to DR had no effect on these results. Impaired DR accumulation, correctable by the addition of 10 milliM azide and deletion of glu-

cose from the medium, was observed in P388/ADR cells. DR accumulation or efflux was not affected by prior exposure of either cell line to TC. Suppression of glycoprotein synthesis altered cell surface properties but had no effect on DR transport characteristics or drug responsiveness. Enhanced glycosylation of cell surface components was associated with development of drug resistance patterns in P388/ADR, but was not a determinant of such resistance. (25 Refs)

CELL ADHESION

23. ADRIAMYCIN-INDUCED CHANGES IN THE SURFACE MEMBRANE OF SARCOMA 180 ASCITES CELLS.

Murphree SA, Tritton TR, Smith PL, Sartorelli AC
Dept. Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale Univ. Sch. Medicine, New Haven, CT, 06510
Biochim Biophys Acta; 649(2):317-324 1981

Adriamycin increases (a) the rate of agglutination of Sarcoma 180 cells by concanavalin A after brief exposure of 2-3 hr and (b) membrane fluidity as measured by ESR within 30 min of exposure at concentrations of the anthracycline at 10(-7)-10(-5) M. The effect of adriamycin on agglutination is not due to an increase in the number of surface receptors for concanavalin A, since the extent of binding of the lectin is not altered by adriamycin and no change occurs in the rate of occupancy of the concanavalin A binding sites by the lectin in cells treated with the antibiotic. The order parameter, a measurement of membrane fluidity, decreases in cells exposed to adriamycin and is dose-related. The results indicate that adriamycin can induce changes in the surface membrane of Sarcoma 180 cells within a brief period of exposure to a low but cytotoxic level of this agent. (Author abstract) (39 Refs)

OTHER RELATED STUDIES

24. INTERACTION OF PHORBOL ESTERS WITH LIPID BILAYERS: THERMOTROPIC CHANGES IN FLUORESCENCE POLARIZATION, PHASE TRANSITION AND CALCIUM IONOPHORESIS.

Deleers M, Defrise-Quertain F, Ruysschaert JM, Maiaisse WJ
Lab. Experimental Medicine, Brussels Univ., 115 Bd de Waterloo, Brussels, Belgium
Res Commun Chem Pathol Pharmacol; 34(3):423-439 1981

The influence of phorbol esters upon the thermotropic behavior of multilamellar liposomes formed of dipalmitoylphosphatidylcholine (DPPC) or dimyristoylphosphatidylcholine (DMPC) was investigated, as a model for possible interferences of the phorbol esters with the phospholipid domain of biological membranes. Both biologically active (TPA, 12-O-tetradecanoylphorbol-13-acetate, PDD, phorbol-12,13-didecanoate) and inactive (4alpha-PDD, 4alpha-phorbol-12,13-didecanoate) phorbol esters lowered the temperature required to cause a fall in fluorescence polarization of a fluorescent probe inserted in the lipid matrix of the DMPC or DPPC liposomes and facilitated the process of calcium exchange-diffusion in DPPC liposomes containing the ionophore A23187. Both of these effect could be due to a decrease in viscosity of the liposomal matrix. However, differential scanning calorimetry revealed that the thermotropic changes evoked by each of these phorbol esters were not identical. In most cases, the phorbol esters decreased both the main phase transition temperature and enthalpy of melting. However, when TPA was incorporated in DMPC liposomes, ie when a myristoyl chain was present in both the phorbol ester and phospholipid, no change in the enthalpy of melting could be detected, whereas the main phase transition temperature decreased in proportion to the TPA content of the liposomes. These findings emphasize the view that phorbol esters indeed interact with phospholipids and that the characteristics of such an interaction may tightly depend on the precise chemical structure of both the phorbol ester and phospholipid under consideration. (Author abstract) (18 Refs)

25. THE IMPORTANCE OF CELL MEMBRANE DEFECTS ON TUMOR CELLS FROM EFFUSIONS IN BODY CAVITIES DURING AND AFTER RADIATION THERAPY AND/OR TUMOR CHEMOTHERAPY.

Hoppe E, Hoppe D
Spezialabteilung fur Tumorzytologie, Geschwulstklinik des Bereiches Medizin, Schumanstr. 20/21, DDR-1040 Berlin, E. Germany
Radiobiol Radiother (Berl); 22(4):484-497 1981

Body-cavity effusions from 30 patients following radiation therapy, chemotherapy, or combined radiation-chemotherapy for tumors and from 40 control patients with various conditions not treated with these therapies were studied. Thirty-nine samples were obtained from the 30 patients in the experimental group (Group 1). Individual treatments and doses varied considerable according to specific disease, patient condition, and other factors. Cellular membrane defects, particularly visible after PAS staining, were found in 31/39 samples from Group 1. No correlation could be demonstrated between individual therapy and membrane defects within Group 1, although sample from patients treated with radiotherapy were obtained after a post-treatment interval approx twice as long as that following chemotherapy. Membrane defects were found in only 1/40 case in the control group. Observed membrane defects were evidently a secondary, nonspecific effect of therapy. Studying such defects should eventually allow reduction in diagnostic uncertainty and determination of the effectiveness of therapy. (26 Refs) (In GER)

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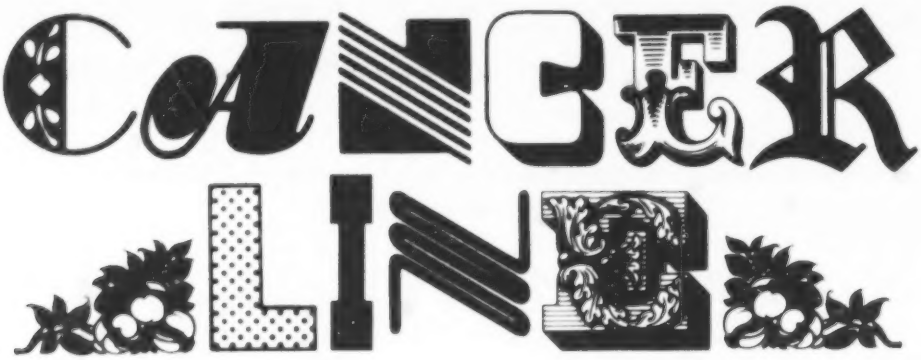

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SERIES CB06 No. 82/06

JUNE, 1982

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

I. HYBRIDOMA MONOCLONAL ANTIBODIES IN THE ANALYSIS OF HUMAN CELL SURFACE ANTIGENS.

Levy R, Bergman Y, Finn O

Dept. Medicine, Div. Oncology, Stanford Univ. Medical Center, Stanford, CA, 94305

In Vitro; 17(12):1051-1057 1981

In the present paper, we will summarize studies we have performed on two distinct human lymphocyte cell surface antigens defined by monoclonal antibodies: Leu-1 and HLA-DR. (Author abstract) (27 Refs)

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2. SPECIFICITY OF SYNGENEIC MONOCLONAL ANTIBODIES AGAINST CELL SURFACE ANTIGENS ON CHEMICALLY INDUCED MOUSE FIBROSARCOMAS.

Klein PA
Tumor Biology Unit, Dept. Pathology, Coll. Medicine, Univ. Florida, Gainesville, FL, 32610
Transplant Proc; 13(4):1762-1764 1981

Development and characterization of 16 monoclonal antibodies (McAb) against tumor-specific transplantation antigens (TSTA) are reported. McAb were developed by immunizing C57BL/6 and BALB/c mice with their own syngeneic, chemically induced fibrosarcomas bearing unique TSTA; the formation of hybridomas followed. Radioimmunoassays were carried out with a large panel of normal cells, tumor cells, and ecotropic and xenotropic murine leukemia viruses (MuLV). Competitive binding assays used tumor cells and MuLV against well-characterized anti-MuLV gp70 and p15(E) McAb. No highly restrictive tumor specificity was found for any of 16 McAb tested. All McAb showed either MuLV-dependent or MuLV-independent cross-reactivity with several mouse tumor strains. Results obtained with five McAb are tabulated. Reactions to anti-MuLV indicated that M3, PC11, and CS-1 tumor strains express gp70 and p15(E) antigens of ecotropic and/or xenotropic viruses on the cell surface. McAB L1 and 4B1 were specific for tumors bearing MuLV antigens. The 4B1 McAb was specific for a molecule similar or identical to the gp70a epitope of ecotropic MuLV. Three McAb showed tumor reactions independent of MuLV antigens. Failure to produce a tumor-specific McAb may be due to differences in T and B cell function or to sampling luck. (9 Refs)

3. IDENTIFICATION OF NEURAL AND ADRENAL MEDULLARY SURFACE MEMBRANE GLYCOPROTEINS RECOGNIZED BY ANTISERA TO CULTURED RAT SYMPATHETIC NEURONS AND PC12 PHEOCHROMOCYTOMA CELLS.

Lee VM, Greene LA, Shelanski ML
Dept. Neuropathology, Univ. Pennsylvania, Phila., PA, 19104
Neuroscience; 6(12):2773-2786 1981

The distribution of glycoproteins in the nervous system and recognized by antisera prepared against cultured rat sympathetic neurons and PC12 pheochromocytoma cells cultured with or without nerve growth factor (+ or-3 NGF) was examined. Antisera prepared against cultured PC12 cells and rat sympathetic neurons recognized a limited number of membrane glycoproteins. Species of glycoproteins recognized by the antisera were qualitatively similar. PC12-and PC12+ antisera immunoprecipitated the same group of fucosylated components. Components included the four recognized by antisera to rat sympathetic neurons. A close qualitative similarity between the antigenic glycoproteins present on PC12+ cells and PC12-cells and on rat sympathetic neurons was observed. NGF greatly increased the relative amount of a trypsin-sensitive, fucose- and glucosamine- containing component, termed NGF-inducible large external (NILE) glycoprotein. Results suggest that rat sympathetic neurons synthesize a material closely resembling NILE glycoprotein. (24 Refs)

4. SURFACE PROTEIN ABNORMALITIES IN LYMPHOCYTES AND PLATELETS FROM PATIENTS WITH WISKOTT-ALDRICH SYNDROME.

Parkman R, Remold-O'Donnell E, Kenney DM, Perrine S, Rosen FS
Div. Immunology, Children's Hosp. Medical Center, Boston, MA, 02115
Lancet; 2(8260):1387-1389 1981

Lymphocytes from three patients with the Wiskott-Aldrich syndrome were subjected to surface radioiodination and their 125I-labeled surface proteins were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Autoradiographs demonstrated the absence in all three patients of an 125I-labeled protein, mol wt 115,000, that was present in normal individuals. In addition, one patient had an additional labeled protein, mol wt 135,000, not found in normal individuals. The platelets from one patient were radioiodinated and the 125I-labeled membrane proteins were analyzed. Glycoproteins Ia and Ib were reduced in amount and restricted in heterogeneity while glycoproteins IIb and IIIa were normal. These results suggest that the primary defect in the Wiskott-Aldrich syndrome may be abnormalities of (glyco)proteins normally present on the surface of lymphocytes and platelets. (Author abstract) (15 Refs)

5. MEMBRANE PROTEINS IN HUMAN SKIN TUMORS.

Landi FL, Cardillo A, Fabrizi G, Farallo A, Miani N, Serri F
(c/o Serri) Dept. Dermatology, Catholic Univ., Largo A. Gemelli 8, I-00168 Rome, Italy
Arch Dermatol Res; 271(4):419-427 1981

The microsomal, mitochondrial, and nuclear fractions of 50 cutaneous tumors, chromatographed on sodium dodecyl sulfate-acrylamide slab gels, revealed electrophoretic profiles (EP) of microsomal proteins which were characteristic of some tumors. Banding patterns of membrane proteins from tumor cells indicated molecular weights around 11,700 daltons and 25,000-40,000 daltons. While normal skin showed relatively simple and reproducible EP of microsomal proteins, those of skin tumors contained more bands of proteins, with qualitative and quantitative differences. Squamous cell and intermediate epitheliomas showed similar EP, which may indicate a relationship between the two cancers. Banding patterns of melanotic melanomas tended to form clusters similar to squamous cell epithelioma, but to a lesser extent. The EP of amelanotic melanoma was different from that of melanotic melanomas. The EP of fibrohistiocytoma showed some similarities to amelanotic melanoma. Mitochondrial membrane proteins of squamous cell and intermediate epitheliomas and normal skin were similar. Melanotic melanoma showed reproducible EP but with more bands in the range greater than 60,000 daltons than in squamous cell epithelioma and normal skin. The EP of nuclear membrane proteins of all squamous cell epitheliomas were reproducible, but with some qualitative and quantitative variations; EP of intermediate epitheliomas were similar. (9 Refs)

6. LACTOPEROXIDASE-CATALYZED IODINATION OF MEMBRANE PROTEINS IN NORMAL AND NEOPLASTIC EPIDERMAL CELLS.

Brysk MM, Snider JM
Dept. Dermatology, Univ. Texas Medical Branch, Galveston, TX, 77550
J Invest Dermatol; 78(1):24-27 1982

Cell surface proteins of normal human, mouse, and rat cells in primary culture, of human basal cell carcinoma, and of carcinogen-transformed cell lines were examined by lactoperoxi-

dase-catalyzed iodination. Autoradiography was used to record the distribution of label in the polypeptide subunits separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. There was no significant difference in the results for normal cells of human, mouse, and rat. On the other hand, carcinogen-transformed mouse cells had many more labeled polypeptide bands of widely distributed mol wt. The iodination profiles from human basal cell carcinoma cells were much more akin to those from normal cells than to those from carcinogen-transformed cells. Treatment of iodinated cells with proteolytic enzymes visibly altered the polypeptide bands. (Author abstract) (21 Refs)

7. PROTEIN KINASE ACTIVITY AND SUBSTRATES AT THE SURFACE OF INTACT HELA CELLS.

Kubler D, Pyerin W, Kinzel V
Inst. Experimental Pathology, German Cancer Res. Center, D-6900 Heidelberg, W. Germany
J Biol Chem; 257(1):322-329 1982

Evidence is presented for the location at the surface of HeLa cells of a protein kinase capable of phosphorylating surface as well as extracellular (foreign) proteins. The reaction products have been found to be proteins containing phosphoryl groups as monoesters of seryl and threonyl residues (but not of tyrosine). The enzyme is of the cyclic AMP-independent type, since neither cyclic AMP nor the heat- and acid-stable inhibitor protein (specific for cyclic AMP-dependent protein kinases) influenced its activity. Further, co-substrate ATP could in part be substituted by GTP, and the spectrum of proteins phosphorylated by the ecto-enzyme differed from that phosphorylated by cyclic AMP-dependent protein kinases. Evidence for the ecto-enzymic nature of this protein kinase includes (a) utilization of co-substrate and location of products at the surface cells carefully controlled as being in an intact state and (b) phosphorylation of exogenous protein (phosvitin; specific serum proteins) by intact cells. Conclusive proof was gained by qualitative and quantitative comparative studies of phosphorylation in cultures with varying degrees of damaged cells either as a whole or after separation into groups of intact and damaged cells by electronic cell sorting. The results of experiments with cell sonicates excluded the possibility that either enzyme or substrates released from damaged cells were simply adsorbing to the cell surface. (Author abstract) (47 Refs)

8. ALTERATION OF MEMBRANE-ASSOCIATED ENZYMES IN DRUG-RESISTANT SUBLINES OF MOUSE LYMPHOBLASTOMA L5178Y CELLS (MEETING ABSTRACT).

Sugimoto Y, Nishimura T, Suzuki H, Tanaka N
University of Tokyo, Tokyo, Japan
Proceedings of the Japanese Cancer Association, 40th Annual Meeting, October 1981, Sapporo, Japan. The Association 415 pp., 1981.

Activities of marker enzymes for various cell components were studied with extracts of adriamycin-, aclacinomycin A- and bleomycin- resistant cells. Alkaline phosphodiesterase activities in all the resistant sublines were higher than that in the parental cells. Na+, K+-ATPase activities of anthracycline-resistant cell lines were higher and that of bleomycin-resistant cell line was lower than that of the parental cells. Other enzymes showed similar activities in the resistant cells to those in the parental cells. The current results, concerning changes of membrane-associated enzymes in drug-resistant sublines of L5178Y cells, support the assumption that the resistance is due to alteration of plasma membrane transport system. (no Refs)

9. GLYCOSAMINOGLYCAN COMPOSITION OF HUMAN ORAL CANCER TISSUE.

Pillai S, Sasidharan VK, Khader A, Vasudevan DM
Regional Cancer Center, Medical Coll. Campus, Trivandrum 11, Kerala, India
Indian J Cancer; 18(4):258-261 1981

Changes in glycosaminoglycan (GAG) composition in neoplastic oral tissues were investigated. A significant increase in the amount of hyaluronic acid and the total sulfated GAG content was observed in tumor tissues compared to healthy specimens. Of the sulfated GAGs, all fractions collected via chromatography showed significantly higher concentrations in tumor tissues. Both normal and tumor tissues, however, showed the same pattern of GAGs. When concentration of GAG was expressed in terms of mg DNA, chondroitin-6-sulfate, dermatan sulfate, and hyaluronic acid showed increased levels in tumor tissues while all other GAG fractions were similar to those obtained from normal tissues. The possible association of membrane changes, especially GAG changes, with nuclear alteration is discussed. (23 Refs)

10. STUDY OF ASIALOGANGLIOSIDES ON SURFACE MEMBRANES OF RAT BONE MARROW CELLS AND MACROPHAGES.

Taki T, Takagi K, Kamada R, Matsumoto M, Kojima K
(c/o Matsumoto) Dept. Biochemistry, Shizuoka Coll. Pharmacy, Oshika, Shizuoka, Shizuoka 422, Japan
J Biochem (Tokyo); 90(6):1653-1660 1981

The presence of asialogangliosides on membrane surfaces of rat bone marrow cells and macrophages was studied by an immunological method using anti-glycolipid antibodies with high specificity. The antibodies against N-acetylgalactosaminyl(beta1-4)galactosyl(beta1-4) glucosylceramide (GA2), galactosyl(beta1-3)N-acetylgalactosaminyl-(beta1 4)galactosyl(beta1-4) glucosylceramide (GA1) and fucosyl(alpha1-2)galactosyl(beta1-3)N acetylgalactosaminyl(beta1-4) galactosyl(beta1-4)glucosylceramide (Fuco-GA1) were prepared, and used for cytotoxicity testing and indirect stainin g by the immunofluorescence technique. In the cytotoxicity test, approximately 40-50% of rat intraperitoneal macrophages were killed by each of the anti-glycolipid antisera. This finding was confirmed by the indirect immunofluorescence staining method with the purified anti-GA2, anti-GA1 and also anti-Fuco-GA1 IgG. When a rat was preimmunized with bovine serum albumin, all macrophages induced in ascites fluid were found to possess GA1 by the staining method. Immature granulocytes were stained specifically with the anti-GA2 antibody. Granulocytes, myelocytes, and megakaryocytes were stained with the anti-GA1 antibody. Blastic myelocytes were stained with the anti-Fuco-GA1 IgG, but lymphocytes were not stained with any of these anti-asialoganglioside antibodies. From these observations, free-type cells such as macrophages and bone marrow cells were found to possess asialogangliosides as membrane components. (Author abstract) (28 Refs)

11. THE (NA+ + K+)-STIMULATED ATP-A FROM YOSHIDA SARCOMA ASCITIC TUMOR CELLS - COMPARISON OF THE PROPERTIES OF PARTIALLY PURIFIED AND HIGHLY PURIFIED ENZYME.

Zaheer A, Braganca BM

Dept. Biochemistry, Univ. Iowa, Iowa City, IA, 52242

Cell Mol Biol; 27(4):387-390 1981

Several properties have been compared in partially purified membrane fragments enriched with respect to (Na+ + K+)-stimulated ATP-A (ATP phosphohydrolase, EC 3.6.1.3) and highly purified enzyme preparations from Yoshida sarcoma ascitic tumor cells. Properties which were compared include, the effect of pH, substrate concentration, Na-K activation and extent of inhibition by a cytotoxic protein (P6) isolated from cobra (Naja naja) venom. All the properties studied were remarkably similar for both forms of the enzyme indicating that the protein structure of the purified enzyme had apparently remained unchanged. (Author abstract) (11 Refs)

12. BETA-NAPHTHYLAMIDASE ACTIVITY OF THE CELL SURFACE OF EHRLICH ASCITES CELLS. REVERSIBLE CONTROL OF ENZYME ACTIVITY BY METAL IONS AND THIOLS.

Short AK, Steven FS, Griffin MM, Itzhaki S

Dept. Medical Biochemistry, Univ. Manchester, Stopford Building, Manchester, England

Br J Cancer; 44(5):709-716 1981

Ehrlich ascites tumor cells grown in mice have a cell-surface trypsin-like neutral protease (TLNP) which is not inhibited by high-mol-wt inhibitors of trypsin. This enzyme is inhibited by low concentrations of zinc, which may be removed by chelating agents, with the consequent return of enzymic activity. Gold, provided as the drugs aurothiomalate or auranofin, also inhibits TLNP. The gold can be removed from the enzyme by incremental addition of thiols. The mechanisms of gold transfer to the active site to cause inhibition and subsequent removal of gold with reactivation of TLNP, have been shown to be controlled by reversible thiol-exchange reactions. (Author abstract) (10 Refs)

13. DIFFERENCE BETWEEN MAGNESIUM AND MANGANESE IONS IN MODIFICATION OF EFFECT OF CATECHOLAMINE ON ADENYLATE CYCLASE SYSTEM IN EHRLICH ASCITES TUMOR CELLS.

Kurokawa T, Kitamura Y, Moriuchi M, Ishibashi S

Dept Physiological Chemistry, Inst. Pharmaceutical Sciences, Hiroshima Univ. Sch. Medicine, Minami-ku, Hiroshima 734, Japan

J Pharmacobio Dyn; 4(10):794-797 1981

Basal and norepinephrine-stimulated adenylate-cyclase activities in Ehrlich ascites tumor cells were measured in the presence of various concentrations of either Mg++ or Mn++. Both activities increased gradually with increase in Mg++ concentration. Both activities were max in the presence of low Mn++ concentrations, and decreased with increase in concentration. The decrease in activity was more pronounced in the norepinephrine-stimulated activity. Similar difference between the two divalent cations was observed in the effect on binding of the beta-adrenergic ligand to the membrane receptor. (13 Refs)

MORPHOLOGICAL STUDIES

14. EFFECT OF ESTRADIOL ON THE ULTRASTRUCTURE OF THE MCF7 HUMAN BREAST CANCER CELLS IN CULTURE.

Vic P, Vignon F, Derocq D, Rochefort H

(c/o Rochefort), U 148, Institut National de le Sante et de la Recherche Medicale, 60 Rue de Navacelles, 34100 Montpellier, France

Cancer Res; 42(2):667-673 1982

We have analyzed the effect of estradiol and of two classes of antiestrogens on the morphology of the MCF7 human breast cancer cell line by scanning and transmission electron microscopy. Estradiol progressively increased the number and the length of microvilli at the cell surface. The density of the microvilli network increased between 2 and 11 days of estrogen treatment, while the cells became more globular and less tightly attached to the surface of the dish. Estradiol also progressively transformed cells into secretory cells containing, at Day 2, large, clear mitochondria and, at Day 4, rough endoplasmic reticulum and Golgi complexes. At Day 6, secretory granules (diameter, 0.2 um), which mainly contained glycoproteins, were first observed in the cytoplasm. By Day 8, they were concentrated at the cell membrane and being liberated into the medium. Larger granules (diameter, 0.8 um), which probably contained lipids, were observed later (Day 11). Cell cultures in 10% fetal calf serum not treated by charcoal contained secretory granules. The modifications were induced by physiological concentrations of estradiol but not 5alpha-dihydrotestosterone. Progesterone (10 nM for 8 days) completely inhibited the effect of estradiol on the microvilli and secretory activity. Tamoxifen or hydroxytamoxifen did not induce secretory activity but did alter the cell morphology compared to control cells. The effects of estradiol were observed in other estrogen receptor-positive breast cancer cell lines (ZR 75-1, T 47 D) but not in an estrogen receptor-negative cell line (BT 2 0). This morphological evidence that estrogens modify the cell surface of breast cancer cells in culture and transform them into 'secretory cells' complements evidence that they induce the release of a glycoprotein with a mol wt of approx 50,000 into the culture medium. (The mol wt was found first to be 46,000. It seems to be closer to 52,000 in a 10% polyacrylamide gel and by using the NEN-labeled proteins as mol wt markers.) (Author abstract) (30 Refs)

15. ULTRASTRUCTURAL ORGAN-SPECIFIC FEATURES OF BREAST CANCER CELLS.

Topuriia IV

Georgian Cancer Res. Center, Tbilisi, USSR

Arkh Patol; 43(11):57-63 1981

Results of electron microscopic examination of 40 surgical specimens of breast cancer are reported. Histologically, 31 patients had infiltrating carcinoma, 2 had medullary carcinoma, 2 had mucinous carcinoma, 1 had lobular invasive carcinoma, 1 had cribriform carcinoma, and 3 had noninvasive intraductal carcinoma in situ. Cells of the different tumors could be divided into two groups: cells in Group 1 had ultrastructural organ-specific features, whereas cells in Group 2 lacked such features. Characteristic organ-specific features included the presence of intracellular tubules and secretory granules; myoepithelial cells were observed in 7/40 specimens. The undifferentiated cells of Group 2 showed a number of tissue-specific features which were indicative of their epithelial origin. The tissue-specific features included the presence of desmosomes, large

ducts, and intercellular tubules with microvilli and basal membranes. (20 Refs) (In RUS)

16. IMMUNOLOGICAL, BIOCHEMICAL, ULTRASTRUCTURAL, AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF A HUMAN GLIOBLASTOMA-DERIVED CELL CULTURE LINE.

Black PM, Kornblith PL, Davison PF, Liszczak TM, Merk LP, Smith BH, McKeever PE, Quindlen EA
Neurosurgical Service, Massachusetts General Hosp., Boston, MA, 02114
J Neurosurg; 56(1):62-72 1982

This report presents the results of a study using multiple techniques of the established human cell line, LM, which has been developed in culture medium from a patient with a right temporoparietal glioblastoma. This cell line has human subtetraploid karyotype and has several features of a transformed line in culture. These include continuous propagation for 10 years, ability to form tumor nodules when transplanted into immunologically suppressed hamsters, and pleomorphic appearance. Ultrastructurally, it is characterized by multiple nuclei, few actin cables, and numerous surface-membrane microvilli, as well as abundant 9-to 10-nm cytoplasmic filaments. By its immunological reactivity, the line can be shown to contain glial fibrillary acidic protein at low levels, consistent with its glial origin and continued nature. Dibutyryl cyclic adenosine monophosphate (db-cAMP) induces formation of long astrocytic-like proc esses as well. Its membrane electrical characteristics include a low resting membrane potential and short time constant. Used in a microtiter antiglioma antibody cytotoxicity assay, LM yields a positive reaction to antibodies in the sera of 80% of patients with astrocytomas and only 9% of normal blood-bank donors, suggesting that it shares common antigens with other astrocytic tumor lines. The varied characteristics of this glioblastoma-derived line emphasize the 'multiforme' nature of the neoplasm and suggest that for characterization of any such line, multiple parameters are necessary to allow comparison with other long-term glioblastoma lines in the literature. The usefulness of the LM line in in vitro cell biological, immunological, chemotherapeutic, and radiobiological studies of gliomas makes such efforts very worthwhile. (Author abstract) (58 Refs)

17. MEMBRANE PROTEIN REDISTRIBUTION DURING DIFFERENTIATION OF CULTURED HUMAN ERYTHROLEUKEMIC CELLS.

Hunt RC, Marshall LM
Dept. Biochemistry, Univ. Mississippi Medical Center, Jackson, MS, 39216
Mol Cell Biol; 1(12):1150-1162 1981

Human erythroleukemic (K562) cells incubated with 0.05 milliM hemin undergo differentiation along the erythroid pathway. RBC proteins synthesized before and after such induction, morphology of the cells and localization of membrane proteins at the cell surface were examined. In the presence of hemin, cells continued to proliferate; after a 24-48 hr delay they started to synthesize Hb. During differentiation, no changes in major cell surface proteins were detected by lactoperoxidase-catalyzed iodination. Specific immune precipitation showed no change in synthesis of spectrin, the major cytoskeletal protein of the mature RBC. While no major changes in cell surface proteins were detected, scanning and transmission electron microscopy revealed marked changes in the morphologic organization of the cell membrane. A process similar but not identical to the enucleation observed in erythroid differentiation in vivo occurred: a smooth-surfaced cell approx. 10 micron in diameter divided from the nucleus-containing part of the cell. Transmission electron microscopy showed that with the exception of ribosomes, these reticulocyte-like cells contained no organelles, but they contained much of the parent cell's Hb, spectrin, and glycophorin. (32 Refs)

18. ULTRASTRUCTURAL HISTOCHEMISTRY OF ORAL LEUKOPLAKIA IN RELATION TO TONOFILAMENT ORIENTATION.

Kandarkar SV, Veturkar PL, Sirsat SM
Cancer Res. Inst., Tata Memorial Centre, Parel, Bombay 400 012, India
Indian J Cancer; 18(4):235-240 1981

A study suggesting a relationship between the morphologic dissociation of tonofilaments and the development of oral leukoplakia is reported. A silver methenamine technique, modified for ultrastructural localization of prekeratin and keratin, was used to make electron histo-chemical observations in 15 biopsy specimens. A positive silver reaction was seen on the plasma membrane of most epithelial cells, desmosomes and tonofilaments. The silver reaction was strongly positive on desmosomes. Randomly oriented and scattered intracytoplasmic tonofilaments are often seen in various oral disorders such as keratoacanthoma, smoker's keratosis, nicotiana stomatitis, etc. Two significant alterations in oral disorders are (1) detachment of desmosomes from the plasma membrane and their internalization or externalization, and (2) detachment of tonofilaments from desmosomes which are then seen intra-cytoplasmic. Dyskeratosis observed in oral leukoplakia, a precancerous condition, could be due to the unusual intra-cytoplasmic location of desmosomes. (20 Refs)

19. HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES IN RAT KIDNEY AFTER CRYOSURGERY.

Sindelar WF, Javadpour N, Bagley DH
Surgery Branch, NCI, NIH, Building 10, Room 10N-116, Bethesda, MD, 20205
J Surg Oncol; 18(4):363-379 1981

Effect of cryoinjury to normal rat kidney was studied at the light and electron microscope level, and characterization of the time course of pathologic changes in kidney tissue after cryosurgery was described from 1 hr up to 14 days after production of a standard cryolesion. Cryoinjury to the kidney resulted in sharply demarcated lesions with minimal inflammatory response during the period of cell destruction and healing by fibrosis. Cellular protein was completely coagulated within 1 hr after cryoinjury, with crystallization of protein and disruption of membranes. Cellular disruption was rapid, and the clearance of cellular debris from the area of injury by macrophage infiltration was accomplished through a quite mild inflammatory reaction. Supporting stromal elements of renal parenchyma remained intact, including collagen tubular framework and basement membranes, and formed the lattice over which fibrosis developed. (Author abstract) (9 Refs)

20. ANALYSIS OF GAP JUNCTIONS AND FORMATION PLAQUES BETWEEN REAGGREGATING NOVIKOFF HEPATOMA CELLS.

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Dept. Genetics and Cell Biology, Univ. Minnesota, St. Paul, MN, 55108
J Ultrastruct Res; 77(3):263-276 1981

Quantitative electron microscopic studies were conducted on freeze-fractured specimens of Novikoff hepatoma cells (N1S1-67) that had previously been dissociated by mechanical agitation in EDTA for 30 min. Membrane interfaces were categorized as positive if they had junctional structures containing formation plaques [FP(+)'s] with gap junctions, and negative those without them FP(-)'s. Both FP's were tallied afer reaggregation times of 5, 15, 30, and 60 min. New FPs appear between 5 and 15 min, and gradually increase in number between 15 and 30 min. FP(-) interfaces devloped more rapidly than FP(+) interfaces initially, and then declined while FP(+) interfaces continue to increase, probably due to conversion of FP(-)'s into FP(+)'s. The number of 9-11 nanometer (nm) particles in FPs per plaque and per interface, and their densities generally increased during the recovery period. FP(+)'s, especially in the 15-min samples, exhibited a relatively constant density of unaggregated 9-11 nm particles, but the density of aggregated particle was highly variable. An analogy was made between 9-11 nm particles in FP(+)'s and the behavior of a substance in a saturated soln. (21 Refs)

21. GAP JUNCTIONS BETWEEN NOVIKOFF HEPATOMA CELLS FOLLOWING DISSOCIATION AND RECOVERY IN THE ABSENCE OF CELL CONTACT.

Preus D, Johnson R, Sheridan J
Dept. Genetics and Cell Biology, Univ. Minnesota, St. Paul, MN, 55108
J Ultrastruct Res; 77(3):248-262 1981

The dissociation of Novikoff hepatoma cell (N1S1-67) was accomplished with EDTA (30 min exposure). Faces of formation plaques (FPs) with and without gap junctions, were observed with electron microscopy of thin-sectioned on freeze-fractured preparations. More than 95% of single cells resulted from the dissociation procedure, but some gap junctions remained. The proportion of membrane faces having any FPs decreased by about half after dissociation, and FPs lacking gap junctions decreased much more than FPs with them. As time passed, after dissociation the blebbing of membranes associated with gap junctions found in a small proportion of dissociated cells disappeared, and the percentage of membrane faces with FPs continued to decline during at least 90 min of post-dissociation recovery. (37 Refs)

22. LABELING OF CELL SURFACE LECTIN RECEPTORS FOR SCANNING ELECTRON MICROSCOPY.

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Dept. Biochemistry, Univ. British Columbia, Vancouver, British Columbia, Canada V6T 1W5
Excerpta Med Int Congr Ser; 545:23-39 1981

Labeling techniques used in scanning electron microscopy for the study of distribution of lectin receptors on cell surfaces are reviewed. Lectins as cell surface specific ligands are discussed. Scanning electron micrographs are provided for illustration. (81 Refs)

MEMBRANE RECEPTOR/ BINDING SITES

23. MONOCLONAL ANTIBODIES AGAINST RECEPTOR FOR EPIDERMAL GROWTH FACTOR INDUCE EARLY AND DELAYED EFFECTS OF EPIDERMAL GROWTH FACTOR.

Schreiber AB, Lax I, Yarden Y, Eshhar Z, Schlessinger J
Dept. Chemical Immunology, Weizmann Inst. Science, 76100 Rehovot, Israel
Proc Natl Acad Sci USA; 78(12):7535-7539 1981

Mice were immunized with human epidermoid carcinoma cells (A-431 cell line) that possess an unusually high number of membrane receptors for epidermal growth factor (EGF). Spleen cells from these mice were fused with NSI cells, a non-secreting murine myeloma. The immunoglobulins secreted by the ob tained hybridomas were screened for specific binding to A-431 cells and selected according to their ability to inhibit the binding of radiolabeled EGF to the membrane of A-431 cells. Several antibodies secreted by cloned hybrid lines were found to inhibit the binding of radiolabeled EGF to membrane receptors of living A-431 cells, human foreskin fibroblasts, and mouse 3T3 fibroblasts and also to membrane preparations from A-431 cells. These monoclonal antibodies induced the early and delayed biological effects mediated by EGF. Like EGF, the antibodies induced morphological changes in A-431 cells and enhanced the phosphorylation of endogenous membrane proteins in membranes from these cells. They also stimulated DNA synthesis in human foreskin fibroblasts. These observations support the notion that the biological information of the EGF-receptor complex resides in the membrane receptor. Furthermore, the antibodies offer a powerful tool to study the structure, processing, and mode of action of EGF receptors. (Author abstract) (34 Refs)

24. CORRELATION BETWEEN THE LOSS OF THE TRANSFORMED PHENOTYPE AND AN INCREASE IN SUPEROXIDE DISMUTASE ACTIVITY IN A REVERTANT SUBCLONE OF SARCOMA VIRUS-INFECTED MAMMALIAN CELLS.

Fernandez-Pol JA, Hamilton PD, Klos DJ
Nuclear Medicine Lab., Veterans Admin. Medical Center, St. Louis, MO, 63125
Cancer Res; 42(2):609-617 1982

We have studied the effects of paraquat (methyl viologen), a herbicide that increases intracellular production of superoxide radical, on the viability of virus-transformed and nontransformed normal rat kidney (NRK) cells in culture. We have shown that a low concentration of paraquat (12.5 uM) is cytotoxic toward virus-transformed cell lines, including Kirsten sarcoma virus- and SV40-transformed NRK cells. The corresponding untransformed NRK cells were resistant to the same and a four-fold higher concentration of paraquat. There was good correlation between the susceptibility of transformed and untransformed cells to paraquat cytotoxicity and their ability to increase the superoxide dismutase (SOD) enzymatic activity. We found that paraquat is cytotoxic toward Kirsten sarcoma virus-transformed and SV40-transformed NRK cells which showed low intracellular SOD activity. The relationship between SOD activity and paraquat cytotoxicity was strengthened by the finding that the tolerance of NRK cells to the drug was associated with high intracellular SOD activity. This report also describes the isolation of a revertant (revertant RE8G3) cell line derived from Kirsten sarcoma virus-transformed NRK cells after paraquat treatment which contains

SOD activity at levels much higher than those found in NRK cells. This revertant is undistinguishable from NRK cells with respect to its lack of transformed cell properties. Not only are these cells normal morphologically but also they do not grow in soft agar, an in vitro property tha t closely correlates with in vivo tumorigenicity. Several biological and biochemical properties of RE8G3 cells, including growth characteristics, *surface receptors* for both transferrin and epidermal growth factor (EGF), and the EGF-dependent 32P phosphorylation of specific membrane polypeptides have been studied. The most interesting conclusion that can be drawn from these studies is that there is a correlation between loss of the transformed phenotype and an increase in both EGF receptors and EGF-dependent 32P phosphorylation of a mol wt 170,000 membrane-associated protein. (Author abstract) (31 Refs)

25. ISOLATION AND CHARACTERIZATION OF C3B RECEPTOR-LIKE MOLECULE FROM MEMBRANES OF A HUMAN B LYMPHOBLASTOID CELL LINE (RAJI).

Barel M, Charriaut C, Frade R
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FEBS Lett; 136(1):111-114 1981

A 140,000 mol wt glycoprotein carrying binding activity of the large fragment of trypsin-cleaved native third component of complement (C3b) was solubilized from the membrane of human B lymphoblastoid cell line, Raji. Some of the properties of this membrane component were analyzed. No change in mol wt of C3b-binding molecule was seen when sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis was performed in the presence of a reducing agent. Based on this results the presence of disulfide-linked subunits was ruled out. The presence of a carbohydrate moiety on this C3b-binding molecule was supported both by its radio-C labeling after intracellular labeling with 14C-glucosamine and by its properties of binding to Lens culinaris. No phosphorylation of the 140,000 mol wt C3b-binding molecule occurred after in vivo labeling of Raji cells by inorganic 32P and in vitro labeling of Raji membranes by gamma-32P-ATP before solubilization of membrane components. The ligand binding activity of this membrane component was tested taking advantage of its elution from C3b-Sepharose beads by acetic acid followed by immediate neutralization to pH 7.4; the acid-eluting C3b-binding protein was able to rebind to new C3b-Sepharose beads and could be eluted from the second affinity material by unlabeled C3b. It was suggested that partial denaturation occurred during the obligatory acid elution procedure and that the 140,000 mol wt glycoprotein extracted from Raji membrane and carrying a C3b binding affinity interacts as a C3b receptor and not as a C3d receptor. (20 Refs)

26. INTERACTIONS OF THE THIRD COMPONENT OF HUMAN COMPLEMENT (C3) WITH TUMOUR CELLS: EVIDENCE FOR PRESENCE OF C3B ACCEPTOR SITES AND DIRECT ACTIVATION OF C3 BY TUMOUR CELLS.

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Ann Immunol (Paris); 132(3):339-350 1981

Interaction of eight human tumor cell lines (Raji and Jijoye: derived from patients with Burkitt's lymphoma; IgR22, IPC501, and Daudel: malignant melanoma; HEP: epidermoid carcinoma; HeLa: epithelial carcinoma; and RD: rhabdomyosarcoma) with the third component of complement (C3) was studied. Incubation of cells with fresh human serum and with fluorescein isothiocyanate (FTIC) anti-C3c induced membranal staining in approx 100% of HEP and HeLa cells and in 20% of IgR22 and IPC501 cells. No fluorescence was observed when the cells were incubated with FTIC anti-C3c alone or with C3-depleted serum. Raji, Jijoye, and Daudel cell lines were incubated with EDTA, an inhibitor of classical and alternative complement pathways. EDTA decreased but did not inhibit the binding of C3b to the cell membrane. Thus, the cells were able to activate C3 directly. This phenomenon was confirmed through the use of purified C3. Fixation of C3 was inhibited only when EDTA and epsilon-aminocaproic acid were added to the medium, indicating the presence of protease activity. This activity was confirmed by varying the concentrations of the reagents. Activation of C3 was enhanced by increasing cell concentration and decreasing inhibitor concentration. Addition of albumin to the C3 solution inhibited the fixation of C3. C3 bound to the tumor cell membrane through its labile binding site when it was directly activated by tumor cells. Cell-bound C3b was not cleaved by tumor cell proteases since no light fragment was observed in the gels. No immune adherence was observed when C3 was deposited directly onto tumor cells. Results suggested that protease activity may be the initial event for activation of the alternative pathway by tumor cells. (20 Refs)

27. DESCRIPTION OF A SIMPLE, SPECIFIC, AND SENSITIVE TEST FOR THE DETECTION OF DETERGENT-SOLUBILIZED C3B RECEPTOR.

Dierich MP, Ehlen T, Mussel HH
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Immunobiology; 160(2):241-247 1981

The C3b receptor was isolated from detergent-solubilized human erythrocyte membranes by a previously described technique. The receptor glycoprotein was shown to enhance EAC14oxy23b rosette formation with Raji lymphoblastoid cells. This provided a specific and sensitive test to detect the solubilized C3b receptor either in crude or highly purified form. The property of the C3b receptor tested by this assay appears to be analogous to properties of beta1H. (Author abstract) (12 Refs)

28. ASSOCIATION OF IMPAIRED IMMUNE RESPONSIVENESS OF LYMPHOCYTES FROM ANIMALS BEARING LARGE TUMORS WITH A MEMBRANE-BOUND SUPPRESSIVE SUBSTANCE.

Wang BS, Heacock EH, Onikul SR, Chang-xue Z, Young TH, Mannick JA
Chemotherapy Res. Dept., Lederle Lab., The American Cyanamid Co., Pearl River, N Y, 10965
Cancer Res; 42(2):416-420 1982

The immunoreactivity of lymphocytes from mice bearing large tumors was investigated. As compared to normal lymphocytes, lymphocytes from tumor bearers manifested a lessened proliferative response to T-cell mitogens and were less capable of destroying syngeneic tumor target cells. However, the impaired reactivity of these lymphocytes was improved significantly after washing repeatedly in tissue culture medium in vitro. The increase in cytolytic activity was tumor specific. A membrane-associated suppressive substance was detected in the washing medium which inhibited the cytotoxicity of washed lymphocytes. The effect of suppressive substance was tumor specific and sensitive to treatment with protein A and anti-immunoglobulin antibody, indicating that the suppressive

substance contained immunoglobulin. The present findings suggest that a membrane-associated suppressive substance(s) may be in part responsible for the diminished immune responsiveness of lymphocytes from animals bearing large tumors. (Author abstract) (23 Refs)

29. EFFECTOR CELL SENSITIVITY TO SUGAR MOIETIES. I. INHIBITION OF HUMAN NATURAL KILLER CELL ACTIVITY BY MONOSACCHARIDES.

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Dept. Basic and Clinical Immunology and Microbiology, Medical Univ. South Carolina, Charleston, SC, 29425
Immunobiology; 160(2):248-258 1981

Human natural killer (NK) cells recognize multiple target antigens. The ligands (antigens) involved in the effector-target cell interaction have not been extensively identified. In the present study, assays of NK activity in the presence of a panel of monosaccharides demonstrated inhibition of cytolysis in a dose-response fashion. We proposed that NK cell activity involves the recognition of carbohydrate structures on target cells via receptors on the effector cell surface. (Author abstract) (23 Refs)

30. STIMULATED MACROPHAGES EXPRESS A NEW GLYCOPROTEIN RECEPTOR REACTIVE WITH GRIFFONIA SIMPLICIFOLIA I-B4 ISOLECTIN.

Maddox DE, Shibata S, Goldstein IJ
to/o Goldstein) Dept. Biological Chemistry, Univ. Michigan, Ann Arbor, MI, 48109
Proc Natl Acad Sci USA; 79(1):166-170 1982

This paper presents data on reactions of murine macrophages with a variety of lectins, with special focus on Griffonia simplicifolia I-B4 isolectin, the only lectin we tried that distinguishes stimulated macrophages from resident populations. Specificity of Griffonia simplicifolia I reaction with carbohydrate determinants at the cell surface is shown by (i) ability of alpha-galactosidase treatment of intact cells to abolish all lectin binding whereas beta-galactosidase has no effect on lectin binding, (ii) ability of methyl alpha-D-galactopyranoside to completely inhibit lectin binding with methyl alpha-D-glucopyranoside having no effect on lectin binding, (iii) ability of brief treatment of intact cells with trypsin to liberate a glycopeptide that reacts with G simplicifolia I to form a precipitate that is dissolved by addition of methyl alpha-D-galactopyranoside or alpha-galactosidase but not by addition of methyl alpha-D-glucopyranoside or beta-galactosidase, (iv) ability of methyl alpha-D-galactopyranoside (but no other monosaccharide) to completely inhibit avid binding of macrophages to G simplicifolia I lectin immobilized on an insoluble support, and (v) ability of immobilized lectin to separate macrophages into highly pure subpopulations of lectin-reactive and lectin-unreactive cells, as shown by examination of fluorescein-labeled lectin-treated cells with phase-contrast/fluorescence microscopy. (Author abstract) (16 Refs)

31. VINBLASTINE AND CYTOCHALASIN B EFFECTS ON DISTRIBUTION OF CONCANAVALIN A RECEPTORS IN NORMAL ATTACHED 3T3 CELLS.

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Inst. Histology and Embryology, Univ. Rome, Rome, Italy
Cell Mol Biol; 27(4):333-339 1981

The effect of the antimicrotubule drug, vinblastine (VB), and of the antimicrofilament drug, cytochalasin B (CB), on the surface distribution of concanavalin A (ConA) binding sites was investigated on normal attached 3T3 cells. When labeled with ferritin-conjugated concanavalin A (Fer-ConA) or with ConA followed by horseradish peroxidase (ConA-HRP), normal 3T3 cells showed a slightly clustered distribution of ConA binding sites. These clusters were distributed randomly over the whole cell surface. This distribution of ConA-receptor complexes was not altered by treatment of cells with CB, despite the marked changes that this drug produced in cell morphology. On the other hand, treatment of 3T3 cells with VB produced marked changes, not only cell morphology, but also in the organization of ConA binding sites: ConA-receptor complexes were cleared from peripheral areas and moved towards the perinuclear part of the cell. The redistribution of ConA receptors is not dependent on VB treatment only, but also needs ConA binding, because in cells treated with VB and fixed before labeling with ConA, the redistribution does not occur. These results are discussed in relation with the role played by microtubules and microfilaments in redistribution of ConA binding sites. (Author abstract) (17 Refs)

32. COMPARATIVE INVESTIGATIONS ON PROLIFERATION KINETICS, CELL SURFACE RECEPTORS, AND HISTOLOGY IN RENAL CELL CARCINOMA (MEETING ABSTRACT).

Klein HO, Dias Wickramanayake P, Klein PJ, Vierbuchen MJ, Ortmann M, Lehmann HD
Medical Clinic, University Cologne, Cologne, W. Germany
UICC Conference on Clinical Oncology, October 28-31, 1981, Lausanne, Switzerland. International Union Against Cancer 131 pp., 1981.

There are only few reports on biological characteristics of hypernephroma. In 64 cases we tried to determine by means of flow-, single cell cytophotometry, in vitro autoradiography (double labeling), analysis of cell surface receptors (lectins +/- neuraminidase pretreatment) and histology as well as case history whether there is a correlation between these parameters and prognosis. Specimen were investigated 10-15 min post operation. They were excised from the marginal and central area of the tumor. The DNA distribution displayed predominantly in 32% a diploid, in 6.890% a hypodiploid, in 13.690% a hyperdiploid, and in 47.7% a heterogeneous tumor population with polyploidic stem lines. The labeling index of tumor cells varied between 5 and 15%. The duration of S-phase was between 10-20 hr. The tumors exhibited a wide range of the quantity and distribution of cell surface receptors (lectin binding sites). Treatment with neuraminidase increased the number of receptor sites in 61% of the cases. Results concerning the correlation of these findings will be presented. (no Refs)

33. HIGH AFFINITY BINDING SITES ON PLASMA MEMBRANE OBTAINED FROM THE LYMPHOBLASTOID CULTURED 1301 CELL LINE FOR HIGHLY RADIOACTIVE SERUM THYMIC FACTOR.

Gastinel LN, Pleau JM, Dardenne M, Sasaki A, Bricas E, Morgat JL, Bach JF
INSERM U 25, Hopital Necker, 161 rue de Sevres, 75015 Paris, France
Biochim Biophys Acta; 684(1):117-126 1982

The interaction of the synthetic serum thymic factor (FTS, facteur thymique serique) with a plasma membrane preparation of human T lymphocytes from the lymphoblastoid T cell line 1301 was studied using 3H-labelled FTS (specific activity 120 Ci/mmol). The binding is temperature dependent and function of the concentration of both 3H-labelled FTS and membrane proteins. At 37 C, using 1 nM of 3H-labelled FTS a steady

state is observed within 80 min. The binding is reversible, specific and saturable. Scatchard analysis reveals the existence of at least two binding sites with respective Kd of the order of 0.516 +-0.2 nM and 110 +-27.8 nM with concentrations of 0.186 +-0.045 pmol and 2.026 +-0.367 pmol per mg of membrane protein. (Author abstract) (36 Refs)

34. HISTAMINE RECEPTORS ON TUMOR CELL LINES (MEETING ABSTRACT).

Wang TB, Quirk J, Whitehead RH, Dempsey PJ, Jose DG
Ludwig Institute, Melbourne, Australia
Clinical Oncology Society of Australia, November 24-17, 1981, Melbourne, Australia. The Society 70 pp., 1981.

Cimetidine is a specific antagonist for the histamine type 2 membrane receptor (H2), but has no apparent effect upon histamine type 1 receptors (H1). There are no reports of histamine receptors on tumor cells. A direct fluorescinated-histamine binding assay has been developed to demonstate H1 and H2 receptors on lymphoid cells. Control sera of fluorescinated albumin, and inhibition of fluorescinated histamine binding free histamine and cimetidine (H2 receptor) were utilized. Using the single cell fluorescent binding method histamine receptors on RIII mouse mammary tumor cells and several human tumor cell lines have been demonstrated. Results showed that histamine receptors could be found on all these tumor cells and in some a proportion of receptors could be blocked by cimetidine. Histamine binding occurred on 90% -100% of cells with no cimetidine blockade in RIII mammary tumor cells, PMC9, human breast carcinoma; RPMI 7932, human melanoma; and COLO 237 melanoma. Cell lines showed H2 receptors in 2% - 6% of cells were PMC123 mesothelial cells from pleural effusion; MM96 melanoma; BEN lung; and T47D breast. Higher proportions of cells with H2 receptors were found on cell lines MB231, breast (51%); PMC42, breast (42%); COLON 463, colonic carcinoma (45%); and MM127 melanoma (22%). (no Refs)

35. EXPRESSION AND MODULATION OF VIRUS RECEPTORS ON LYMPHOID AND MYELOID CELLS: RELATIONSHIP TO INFECTIVITY.

Morishima T, McClintock PR, Billups LC, Notkins AL
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Virology; 116(2):605-618 1982

Using a sensitive and specific assay for the binding of radiolabeled encephalomyocarditis (EMC) virus to cell surface receptors, we have measured the kinetics of virus attachment to murine lymphoid and myeloid cells, and have shown that receptors for this virus vary with the phase of cell growth and can be modulated or, in some cases, induced. The initial rate constant (KR) for resident peritoneal macrophages was 0.8 X 10(-9) cm3 min-1 cell-1, but after in vivo stimulation the rate of binding increased by a factor of 2. In contrast, unstimulated splenic T and B lymphocytes failed to bind EMC virus, but after exposure to appropriate mitogens, the initial rate constant increased to as high as 6.7 x 10(-9) cm3 min-1 cell-1. Studies with continuous cell lines showed that the binding of virus to receptors varied by up to 10-fold and was greatest during the exponential growth phase as compared to the stationary phase. Although the KR increased following exposure of lymphocytes to mitogens, immunofluorescence revealed that only a small fraction of the cells actually contained viral antigens, suggesting that virus receptors either were induced in only a small subpopulation of lymphocytes or that postattachment restriction prevented viral replication in a large fraction of the cells. A survey of six cloned BALC/c T and B cell lymphomas arrested at different stages of differentiation showed that two of these lymphomas possessed EMC virus receptors, arguing that receptors are present only at certain stages of cell differentiation. Evidence that receptors are functionally important comes from experiments which showed that only cells with a KR greater than 0.1 x 10(-9) cm3 min-1 cell-1 are susceptible to infection. These studies suggest that factors which influence the expression and modulation of virus receptors may determine the outcome of certain viral infections. (36 Refs)

TRANSPORT STUDIES

36. IMMUNOLOGICAL DELINEATION IN NORMAL AND MALIGNANT CELLS OF A MEMBRANE PROTEIN INVOLVED IN GLUCOSE TRANSPORT. I. PREPARATION AND PROPERTIES OF THE ANTIBODY.

Gingrich RD, Wouters M, Bramwell ME, Harris H
Sir William Dunn Sch. Pathology, South Parks Road, Oxford OX1 3RE, England
J Cell Sci; 52:99-120 1981

Partially purified preparations of the 200,000 Mr membrane glycoprotein described by Bramwell and Harris were used as antigens to generate monoclonal antibodies. Whether the protein was prepared from human or mouse tumors, the mice immunized with it yielded monoclonal antibodies of broadly similar specificities. All the antibodies obtained were of the immunoglobulin M class, despite a prolonged immunization schedule, and recognized an antigen that was present on the surface of mouse, rat and human cells. One such antibody, M/27, was studied in detail. With the exception of two rat kidney cell lines, the antigen detected by M/27 was found on the surface of all cells grown in culture, whether malignant or not. However, in the mouse, the species in which the antibody was generated, M/27 discriminated sharply between malignant tumors and normal tissues excised directly from the animal. In normal adult or embryonic mouse tissues, whether they were composed of multiplying cells or not, the antigen detected by M/27 was present on the cell surface only at the lower limit of detection, if at all. Malignant mouse tumors grown in the animal produced large amounts of the antigen. Explanation of embryonic mouse fibroblasts induced the expression of the antigen, which was not detected on these same cells in their normal habitat in the growing embryo. Conversely, non-malignant cells that express the antigen in vitro cease to do so when they are re-introduced into the animal. The antigenic determinant appeared to be polypeptide in nature rather than polysaccharide. (Author abstract) (40 Refs)

37. IMMUNOLOGICAL DELINEATION IN NORMAL AND MALIGNANT CELLS OF A MEMBRANE PROTEIN INVOLVED IN GLUCOSE TRANSPORT. II. FUNCTION OF THE ANTIGEN.

Gingrich RD, Wouters M, Bramwell ME, Harris H
Sir William Dunn Sch. Pathology, South Parks Road, Oxford OX1 3RE, England
J Cell Sci; 52:121-135 1981

A review of the literature suggested that the antigen detected by the M/27 antibody described in the preceding paper might be involved in the transport of glucose across the cell membrane. This idea was tested by measuring the amount of

M/27 antibody bound to cells under a range of conditions known to influence the rate of glucose uptake. Glucose starvation and the administration of insulin under appropriate conditions are known to increase the Vmax for glucose uptake in certain cells, and high cell de nsity in vitro is known to decrease the Vmax. Glucose starvation was found to increase the amount of M/27 antigen present on the surface of two malignant cell lines, but produced no detectable change in five different non-malignant cell types. Insulin increased the amount of M/27 antigen on the surface of both malignant and non-malignant cells. At high cell density, the amount of M/27 antigen present on the cell surface decreased. The changes in the Vmax for glucose uptake produced by these manipulations have been shown not to require the synthesis of protein. This was also found to be true for the changes in M/27 antigen concentration. By disrupting cells in detergent, it could be shown that there was an intracellular pool of M/27 antigen. The total amount of M/27 antigen in the cell was found to be about 4x as large as the amount of the antigen on the cell surface. This figure agrees with the value given in the literature for the size of the intracellular pool of the glucose transport system. The amount of M/27 antigen on the cell surface was found to vary during the cell cycle. A dramatic increase occurred early in S phase. (Author abstract) (31 Refs)

38. ENHANCED AMINO ACID TRANSPORT IN CULTURED HEPATOCYTES DURING LIVER DEVELOPMENT.

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J Biochem (Tokyo); 90(6):1821-1824 1981

Hepatocytes from neo- and postnatal rats were successfully isolated, purified from non-hepatocytes (erythropoietic cells), and cultured in sufficient amounts to perform transport kinetics of nutrients. In the presence of sodium (Na+-dependent component) the amino acid uptake, measured with alpha-amino [1-14C]isobutyrate (AIB) as a tracer, was markedly enhanced during early postnatal phases. Kinetic analyses of these enhanced velocity data revealed the presence of a low-Km transport system in the range of amino acid concentration in which AIB mimics physiological properties. The system provides neonatal hepatocytes with a great potential for concentrating amino acids during the early postnatal stage, and may have considerable effect on membrane linked regulatory processes during liver development. (Author abstract) (19 Refs)

39. APPARENT METHIONINE AUXOTROPHY OF SOME TUMOUR CELL LINES MAY BE LINKED TO IMPAIRED AMINO ACID TRANSPORT.

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Eur J Cancer Clin Oncol; 17(12):1323-1329 1981

An investigation of the L-methionine requirement of five cell lines in vitro shows an increased dependence on extracellular L-methionine in the order: human embryonic bladder fibroblasts (HE), human bladder carcinoma (EJ), mouse lymphoma (TLX5), mouse bladder carcinoma (MB) and Walker rat carcinoma (W.256). This order correlates with the initial velocity of transport of this amino acid and the ste ady state concentrations achieved. The order of L-methionine requirement is the same as the order of the ability of these cell lines to survive and grow in alpha L-methionine-depleted medium containing only L-homocysteine. The tumor cell lines all have a decreased maximal initial rate of L-methionine transport (Vmax) and a lower Km value than two normal cells investigated (hepatocytes and fibroblasts). Kinetic studies permit the identification of two transport systems for methionine in W 256 and TLX5 and only one for the other cell lines. The initial rate of transport of L-lysine in these cell lines follows the same order as for L-methionine. Studies using L-[methyl-3H]-methionine labeled cells show that, in addition to a defective transport system for methionine in W.256 and MB, the rate of loss of label from nucleic acids and proteins is also higher for these two cell lines. These results suggest that the apparent methionine auxotrophy of some tumor cell lines may be due to an impaired capacity to concentrate methionine and a higher turnover rate. (Author abstract) (20 Refs)

40. EFFECT OF H+ ON THE KINETICS OF NA(+)-DEPENDENT AMINO ACID TRANSPORT IN EHRLICH ASCITES TUMOR CELLS: EVIDENCE FOR H+ AS AN ALTERNATIVE SUBSTRATE.

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J Cell Physiol; 109(3):507-516 1981

The kinetic response of Na+-dependent alpha-aminoisobutyric acid (AIB) transport to H+ was investigated in Ehrlich ascites tumor cells. Initial AIB fluxes were measured in the presence of varying external Na+ and H+ concentrations. Elevation of H+ at both high (154 mEq/liter) and low (10 mEq/liter) of external Na+ concentrations decreased max influx and increased the apparent Km for AIB influx. In the presencea of very low concentrations of Na+ (0.94 mEq/liter), alterations in H+ had no effect on AIB flux. Two kinetic models are proposed which are consistent with the experimental results: (1) an order bireactant (Na+ binding necessary before AIB binding) system or (2) a random bireactant system. Both models require H+ to serve as an alternative substrate for Na+. Test of fit of both models to data from the present study gave similar results. (30 Refs)

41. T-CELL MITOGENS CAUSE EARLY CHANGES IN CYTOPLASMIC FREE CA2+ AND MEMBRANE POTENTIAL IN LYMPHOCYTES.

Tsien RY, Pozzan T, Rink TJ
Dept. Physiology-Anatomy, Univ. California, Berkeley, CA, 94720
Nature; 295(5844):68-71 1982

Effects of concanavalin A, phytohemagglutinin, mitogenic calcium ionophores (A23187 and ionomycin), and the cocarcinogen 12-O-tetradecanoylphorbol-13-acetate on cytoplasmic Ca++ level and membrane potential in mouse thymocytes and pig node lymphocytes are reported. Ca++ was measured with the use of a fluorescent indicator (quin2) that was loaded into the cytoplasm of intact cells. (35 Refs)

MEMBRANE BIOGENESIS AND METABOLISM

42. BIOSYNTHESIS OF CELL SURFACE SULFATED GLYCOPROTEINS BY CULTURED VASCULAR ENDOTHELIAL CELLS.

Heifetz A, Allen D
Dept. Biochemistry, Univ. Texas Health Science Center at Dallas, Dallas, TX, 75235
Biochemistry; 21(1):171-177 1982

Cultured vascular endothelial cells incorporate 35SO4 into a class of oligosaccharides which are N-glycosidically linked to cell-surface or extracellular proteins. This type of sulfated oligosaccharide was not synthesized by smooth muscle cell cultures and many represent sulfation of vascular tissue glycoproteins which are unique to endothelial cells. These endothelial cell 35S-labeled oligosaccharides are not released from the polypeptides under alkaline conditions that cleave O-glycosidically linked chains. The incorporation of both [3H]hexosamine and 35SO4 into these oligosaccharides is inhibited by tunicamycin, an antibiotic which inhibits protein N-glycosylation. 35S-Labeled oligosaccharides, isolated as glycopeptides, were resistant to a variety of chemical and enzymatic treatments which degraded vascular cell sulfated glycosaminoglycans but appear to contain terminal sialic acid residues. Endothelial cell sulfated oligosaccharide chains could also be distinguished from sulfated glycosaminoglycans by the apparent size and charge. Endothelial cells released or secreted 35S-labeled glycoproteins into the culture medium. These soluble 35S-labeled glycoproteins were partially purified by DEAE-cellulose chromatography and separated from the 35S-labeled proteoglycans, which are also released into the medium. The major 35S-labeled glycoproteins released from the cells had apparent molecular wt of 24,000-66,000. The cell-associated 35S-labeled glycoproteins, released with ethylenediaminetetraacetate or extracted with 0.5% octyl glucoside, were partially purified on DEAE-cellulose and had apparent molecular wt of 45,000-250,000. (Author abstract) (41 Refs)

43. MEMBRANE RECYCLING IN SECRETORY CELLS: IMPLICATIONS FOR TRAFFIC OF PRODUCTS AND SPECIALIZED MEMBRANES WITHIN THE GOLGI COMPLEX.

Farquhar MG
Section Cell Biology, Yale Univ. Sch. Medicine, New Haven, CT, 06510
Methods Cell Biol; 23:399-427 1981

Studies in mature plasma and myeloma cells have demonstrated the existence of considerable membrane traffic from the cell surface to Golgi cisternae, secretory vacuoles, and lysosomes comparable to that previously detected in exocrine and endocrine glandular cells. (63 Refs)

44. SIALIC ACID METABOLISM IN NUTRITIONALLY MODULATED MAMMARY GLAND CARCINOGENESIS.

Fox OF, Kishore GS, Carubelli R
Biomembrane Res. Lab., Oklahoma Medical Res. Foundation, 825 Northeast Thirteenth St., Oklahoma City, OK, 73104
Cell Mol Biol; 27(5):473-482 1981

The activities of CMP-N-acetylneuraminic acid synthetase, sialyltransferase and neuraminidase were investigated in female Sprague-Dawley rats with mammary adenocarcinomas induced by oral administration of 7,12-dimethylbenz[a]anthracene. The carcinogenic process was modulated by the levels and degree of unsaturation of the dietary lipids in the presence and in the absence of dietary antioxidants. The activities of the biosynthetic enzymes responsible for the activation and transfer of sialic acids, ie CMP-N-acetylneuraminic acid synthetase and sialyltransferase were higher in tumors than in control mammary glands. The cytosolic and membrane-bound neuraminidases, on the other hand, were higher in control tissue than in tumors. In the low-fat dietary group, which had the lowest rate of tumor incidence, a tocopherol effect on sialic acid metabolism was detected; rats fed low-fat diet supplemented with alpha-tocopherol exhibited the highest levels of control mammary gland sialyltransferase activity, whereas tocopherol deficiency was accompanied by a loss of the tumor-dependent enhancement of CMP-N-acetylneuraminic acid synthetase. The enzyme profiles in the livers of control and tumor-bearing rats, although less well defined, showed some of the general trends observed in mammary tissues. (Author abstract) (21 Refs)

45. MOVEMENT OF PLASMA MEMBRANE PROTEINS OF EHRLICH ASCITES TUMOR CELLS IN RELATION TO CAP FORMATION INDUCED BY CONCANAVALIN A: A STUDY OF THE NON-CAPPED AREAS.

Nakamoto S
Dept. Pathology, Okayama Univ. Medical Sch., Okayama, Japan
Acta Med Okayama; 35(4):221-234 1981

In order to get precise information about the movement of plasma membrane proteins in cap formation, cyto- and biochemical analyses were made of the plasma membranes from non-capped areas of Ehrlich ascites tumor cells (EATCs) exposed to concanavalin A (ConA). Blebs formed by treatment with cytochalasin B (CB) of the non-capped areas of cells having a cap were isolated and used as the plasma membranes from non-capped areas (ConA-CB-bleb fraction). This bleb fraction was compared with a bleb fraction prepared from cells without ConA-treatment (CB-bleb fraction). Cytochemical analysis of ConA-CB-bleb fraction revealed a decrease in ConA binding sites (ConA-BS) compared to the CB-bleb fraction. SDS polyacrylamide slab gel electrophoresis also revealed a decrease in the major components of ConA-BS of the ConA-CB bleb fraction. The minor components of ConA-BS showed no distinct quantitative difference between the ConA-CB-bleb and CB-bleb fractions. Na+, K+ adenosine triphosphatase (ATPase), 5'nucleotidase (5'ND) and gamma-glutamyl transpeptidase (gamma-GTP) did not show any decrease in activity in the ConA-CB-bleb fraction, but the activity of K+ stimulated phosphatase (K-Pase) was decreased. The findings indicate that there are two types of plasma membrane glycoproteins in EATCs; one includes those participating in cap formation due to ConA, eg, the ma jor components of ConA-BS and K-Pase, and the other, those not participating in such cap formation, eg, some minor components of ConA-BS, ATPase, 5'ND and gamma-GTP, which keep their places without moving. (Author abstract) (47 Refs)

CELL ADHESION

46. A GELATIN-BINDING 70,000-DALTON GLYCOPROTEIN SYNTHESIZED DISTINCTLY FROM FIBRONECTIN BY NORMAL AND MALIGNANT ADHERENT CELLS.

Vartio T, Vaheri A
Dept. Virology, Pathology, Univ. Helsinki, Haartmaninkatu 3, SF-00290 Helsinki 29, Finland
J Biol Chem; 256(24):13085-13090 1981

Fibronectin is a high mol wt glycoprotein that has affinity for different types of collagens and especially for denatured collagen (gelatin). This property, characteristic of fibronectin, is widely used as a means of isolation of the protein. We now report on another gelatin-binding glycoprotein, Mr = 70,000, synthesized and secreted by various cultured adherent cells. The Mr = 70,000 polypeptide incorporates both radioactive amino acids and mannose, is monomeric, and contains intramolecular disulfide bridges as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under nonreducing or reducing conditions. Several normal and malignant cell lines of both fibroblastic and epithelial type synthesize and secrete this polypeptide. Of the cell lines studied, only murine neuroblastoma C 1300 cells did not produce it although they produced fibronectin, while the Mr = 70,000 polypeptide is the only gelatin-binding protein produced by BeWo trophoblastic cells. No immunological cross-reactivity was detected between fibronectin and the Mr = 70,000 polypeptide as judged by electrophoretic protein transfer experiments and use of monoclonal anti-fibronectins and specific antisera against fibronectin and the Mr = 70,000 protein. Pulse-chase experiments in fibroblast cultures indicated that the Mr = 70,000 protein is synthesized separately from fibronectin and processed from an Mr = 68,000 precursor polypeptide. Isoelectric focusing showed that the Mr = 70,000 gelatin-binding proteins of different cell lines have identical pI values at 5.3-5.4. The Mr = 70,000 protein bound to gelatin-Sepharose as firmly as fibronectin, and both were eluted with 1 M arginine or 6 M urea but not with 3 M NaCL. The two proteins were separated by gel filtration, indicating that they are not associated with each other in fibroblast culture medium. Furthermore, the Mr = 70,000 protein produced by BeWo cells also binds to gelatin-Sepharose indicating that it binds independently of fibronectin. (Author abstract) (36 Refs)

47. FIBRONECTIN.

Haim S, Gilhar A
No affiliation given
Harefuah; 100(12):584-585 1981

Structure and function of the two-chain polypeptide fibronectin, found in the serum and on cell membranes, are reviewed. In neoplastic processes low levels of the protein have been found: its level decreases when tumor cells are injected into an immunocompromised host. Proposed mechanisms include decreased mRNA, increased proteolysis, decreased membrane binding, increased plasminogen levels, and trapping of fibronectin by tumor cells. (39 Refs) (In HEB)

48. PURIFICATION AND CELL-SPREADING EFFECT OF GLYCOPROTEIN OF SPONTANEOUSLY MALIGNANT FIBROBLASTS.

Adachi T, Midorikawa O
Dept. Pathology, Faculty Medicine, Kyoto Univ., Kyoto, Japan
Cell Mol Biol; 27(5):445-453 1981

Mouse fibroblast cells (m cells) and so-called reticulum cells (MR cells and SP cells), transformed into malignant cells in vitro, were transferred to a serum-free culture medium, and the morphological and functional differences of these two kinds of cells were studied. We found that the fibroblast cells produce a unique glycoprotein which has the biological function of inducing cell-spreading of so-called reticulum cells on the substratum in the serum-free culture medium. This glycoprotein was purified from the conditioned medium of the transformed fibroblast cells. The mol wt of this protein is about 230-250 x 10(3). The so-called reticulum cells themselves do not produce detectable amounts of this protein. Specific antiserum against this glycoprotein shows a single precipitation line against not only this glycoprotein, but also against serum protein. This antiserum inhibits the cell-spreading-inducing activity of this glycoprotein in vitro. Immunofluorescent microscopy shows that this glycoprotein exists on the cell surface and in the cytoplasm of normal and some transformed fibroblast cells. Undifferentiated fibrosarcoma cells, however, do not produce this glycoprotein in vitro. (Author abstract) (24 Refs)

49. EXTRACELLULAR MATRIX PROTEINS IN HUMAN BLADDER TUMORS (MEETING ABSTRACT).

Sakashita S, Hashi H
Department of Urology, Asahikawa Medical College, Asahikawa, Japan
Proceedings of the Japanese Cancer Association, 40th Annual Meeting, October 1981, Sapporo, Japan. The Association 415 pp., 1981.

Fibronectin is a major component of non collagenous glycoproteins in extracellular matrix. Laminin is a relatively new glycoprotein localized in basement membrane. These proteins play a role in cell-cell contacts. We will show the difference of extracellular matrices between normal and malignant bladder tissues. Fibronectin was purified from human plasma by Gelatin-Sepharose. Laminin was purified as a previous report in ABB, 1980. By immunofluorescent technique, laminin was seen in basement membranes both of malignant and normal bladder. But fibronectin was lacking in the cell surface of malignant epithelium. The fact may be the rationale to explain the looseness of cell-cell adhesion in malignant cells as a cause of metastasis. (no Refs)

50. SELECTIVE ADHESION OF EMBRYONAL CARCINOMA CELLS AND DIFFERENTIATED CELLS BY CA2+ -DEPENDENT SITES.

Takeichi M, Atsumi T, Yoshida C, Uno K, Okada TS
Dept. Biophysics, Kyoto Univ., Kyoto 606, Japan
Dev Biol; 87(2):340-350 1981

The specificity of adhesion between embryonal carcinoma cells and fibroblastic cells of various origins was studied. Embryonal carcinoma cells have intercellular adhesion sites requiring Ca++ (CDS). These sites were found to be sensitive to proteases but resistant to them in the presence of Ca++. CDS with a similar protease sensitivity is present in fibroblastic cells. When embryonal carcinoma cells of different lines were mixed, they adhered to each other nonselectively by CDS. Nonselective adhesion by CDS occurred also between fibroblastic cells

of various lines. When embryonal carcinoma and fibroblastic cells were mixed, they preferentially adhered to homotypic cells. Fab fragments of antibodies raised against F9 cells (a nullipotent line of embryonal carcinoma) inhibited the adhesion between embryonal carcinoma cells but not between fibroblastic cells. This inhibitory activity of Fab was absorbed with embryonal carcinoma cells with CDS, but not with fibroblastic cells with CDS or embryonal carcinoma cells from which CDS was experimentally removed. SDS-polyacrylamide gel electrophoresis of radioiodinated cell surface proteins showed that the presence of a 140K-dalton component correlated with the presence of CDS in embryonal carcinoma cells, while the presence of a 150K-dalton component correlated with the presence of CDS in fibroblastic cells. These results suggest that CDS in embryonal carcinoma and fibroblastic cells comprise distinct molecules. (Author abstract) (43 Refs)

OTHER RELATED STUDIES

51. HYBRIDOMAS: A NEW DIMENSION IN BIOLOGICAL ANALYSES.

Kennett RH
Dept. Human Genetics, Univ. Pennsylvania Sch. Medicine, Philadelphia, PA, 19104
In Vitro; 17(12):1036-1050 1981

Since the first report of hybridomas producing monoclonal antibodies by Kohler and Milstein in 1975, this technique has spread to nearly all areas of biological, biochemical, and biomedical research. Watching the use of these methods spread from immunologists to cell biologists, developmental biologists, biochemists and to other biological disciplines and observing the nearly logarithmic increase in publications using these reagents has been in itself fascinating and informative. An overview of the development of this technology and its applications is presented including the use of monoclonal antibodies to study cell surface molecules, differentiation antigens, receptors, and histocompatibility antigens. The use of these antibodies to analyze microorganisms and parasitic antigens as well as their use in the genetic analysis of human cell surface antigens and the detection of polymorphic variation in enzymes and other proteins is discussed. Examples of the application of monoclonal reagents to the study of tumor cell biology including the labeling of metastatic tumor cells and the detection of cell surface molecules implicated in the regulation of growth control and cell division are provided. (Author abstract) (232 Refs)

52. ASSAYS TO MEASURE PLASMA MEMBRANE AND CYTOSKELETAL CHANGES IN INTERFERON-TREATED CELLS.

Pfeffer LM, Wang E, Landsberger FR, Tamm I
Rockefeller Univ., New York, NY, 10021
Methods Enzymol; 79:461-473 1981

Techniques were described for the measurement of interferon (IF)-induced changes in cell size, cytoskeleton, cell surface fibronectin distribution, lateral mobility of cell surface receptors, and fluidity of the plasma membrane. Cell surface area of human diploid fibroblasts (between passages 10-20) was measured with the use of photomicrographs; cell vol was determined by a Coulter H4 Channelyzer. Cells treated with IF were more heterogeneous than control cells as indicated by greater coefficient of variation (21.5% vs 11.5%) of the cell vol distribution. Indirect immunofluorescence staining of microtubules, 10-nanometer filaments, microfilaments, and fibronectin of human fibroblasts was also discussed. Human HeLaS3 cells were used to show the effect of IF on lateral mobility of cell surface receptors for fluorescein-conjugated concanavalin A, which was examined by epifluorescence microscopy. In IF-treated cells incubated at 37 C, the fluorescence remains patchy and does not tend to form a cap, as occurs in control cells. The use of electron spin resonance techniques plus probe molecules (spin labels) to study the influence of IF on structure of the plasma membrane lipid bilayer in HeLaS3 cells and human fibroblasts was also discussed, with comments on some technical details and interpretation of the resulting spectra. (20 Refs)

53. MAXIMIZING DIFFERENCES IN THE CONCANAVALIN A-INDUCED BLASTOGENIC RESPONSES OF LYMPHOCYTES FROM BREAST CANCER PATIENTS AND CONTROLS BY THE USE OF ALPHA-METHYL-D-MANNOSIDE.

Distasio JA, Cheresh DA, Schilder RJ, Vogel CL, Silverman MA, Lopez DM
Dept. Microbiology D4-4, Univ. Miami Sch. Medicine, P.O. Box 016960, Miami, FL, 33101
JNCI; 68(1):69-74 1982

In an attempt to magnify differences in the immune responses of potentially immunosuppressed cancer patients and normal controls, an assessment was made on the effects of the competitive inhibitor alpha-methyl-D-mannoside on the concanavalin A (Con A)-induced blastogenic responses of lymphocytes from each of these populations. Lymphocytes from breast cancer patients with metastatic disease were significantly deficient in their capability to undergo blast transformation regardless of whether the monosaccharide inhibitor was added to the assay cultures. In contrast, lymphocytes from breast cancer patients who did not display metastatic disease were capable of normal blastogenic responses to Con A. The addition of alpha-methyl-D-mannoside to lymphocyte cultures caused a significantly greater inhibition of the blastogenic responses of these patients' cells as compared to cells of normal controls. Thus the monosaccharide seems to serve as a useful reagent for optimizing differences between lymphocyte blastogenic responses of normal donors and those of immunodepressed donors. The results suggest that lymphocytes from breast cancer patients without clinically evident metastases possess some modification of their cell membrane. One possibility discussed was that the number or distribution of receptors for Con A on the membrane of lymphocytes of these patients is deficient. (Author abstract) (27 Refs)

54. ACTIVATION OF HUMAN LYMPHOCYTES BY CONCANAVALIN A OR PURIFIED PROTEIN DERIVATIVE RESULTS IN NO ALTERATION OF FLUORESCENCE POLARIZATION OF LIPID PROBES ALTHOUGH THE ELECTROPHORETIC MOBILITY OF THE CELLS IS CHANGED.

Parola AH, Kaplan JH, Lockwood SH, Uzgiris EE
Dept. Chemistry, Ben-Gurion Univ. of Negev, Beer-sheva 84120, Israel
Biochim Biophys Acta; 649(3):616-624 1981

Upon stimulation with either concanavalin A or the tuberculin antigen, purified protein derivative, human peripheral blood lymphocytes, purified on Ficoll-Hypaque, did not exhibit a concomitant lipid fluidity alteration as measured by fluorescence polarization (P) of the lipid probe, 1,6-diphenyl-1,3,5-hexatriene (DPH). This result was independent of the incuba-

tion period, ranging from 10 min to 72 hr. However, a general reduction in polarization value, from p = 0.287 (maintained for up to 2 hr of incubation) to p = 0.225 after 20 hr was observed for both experimental and control samples. Moreover, fluorescence polarization studies of the nonpenetrating modified DPH cationic lipid probe, 1-[4'-trimethylaminophenyl]-6-phenyl-1,3,5-hexatriene (TMA-DPH), also failed to show any change in lipid fluidity subsequent to a 1-3 hr incubation of lymphocytes with concanavalin A. Cell electrophoretic mobility, however, was altered (mean cell mobility increased by 10-15%) in a fast response to stimulation and was observed within several hours of in vitro application of concanavalin A and purified protein derivative. This initial response disappeared with further incubation at 37 C (greater than 3 hr) and was followed by a decline of cellular mobility of the concanavalin A-exposed cells after 48 and 72 hr of incubation. The unstimulated control cells did not change in mobility as a function of incubation time. The slow decline in mean cell mobility of the experimental cells is believed to be associated with blastogenesis. It is concluded that neither blastogenic transformation nor short term membrane alterations associated with human lymphocyte activation lead to lipid f luidity changes as measured in steady state by the fluorescence polarization of both DPH and TMA-DPH. (Author abstract) (41 Refs)

55. CYTOSKELETAL AND TRANSMEMBRANE INTERACTIONS IN THE EXPRESSION OF TUMOURIGENICITY IN HUMAN CELL HYBRIDS.

Der CJ, Ash JF, Stanbridge EJ
(c/o Stanbridge) Dept. Microbiology, Coll. Medicine, Univ. California, Irvine, CA, 92717
J Cell Sci; 52:151-166 1981

The cytoplasmic cytoskeletal organization was examined by immunofluorescence analysis in a series of paired non-tumorigenic and tumorigenic HeLa/fibroblast hybrids. The microfilament and microtubule complexity in tumorigenic hybrids was compared to that of the corresponding transformed, non-tumorigenic hybrids from which they were derived. Since both the non-tumorigenic and tumorigenic hybrids displayed well-organized microtubules, the disruption of these cytoskeletal elements is not required for transformation or tumorigenicity. In contrast, the microfilament organization was drastically altered in association with the re-expression of tumorigenicity. However, treatment of these tumorigenic segregant lines with sodium butyrate or dexamethasone resulted in a more flattened morphology and the appearance of well-developed microfilament bundles. This indicated that microfilament complexity was related to cellular morphology and not to tumorigenic potential. Finally, results of double immunofluorescence analyses for microfilaments and fibronectin staining in these hybrids agree with the existence of a transmembrane association between these two systems. In both normal fibroblasts and non-tumorigenic hybrids a close, but not identical, distribution was seen for surface fibronectin and cytoplasmic microfilaments. In the tumorigenic hybrids the lack of a well-organized microfilament system was associated with a much reduced stitched-like pattern for fibronectin. The antibody-induced redistribution of surface beta2-microglobulin reflected this loss of a transmembrane influence on the organization of surface components. Unlike the non-tumorigenic hybrids, the tumorigenic segregants possessed an altered extracellular matrix, rounded morphology, and reduced adhesion capabilities. The corresponding reduction in microfilament organization in these hybrids suggests that there is a close interrelationship between these cellular parameters. The exact contribution of these alterations to tumorigenic potential is unclear but the apparent loss of the transmembrane interaction with the cell surface organization may well represent a crucial alteration central to in vivo growth potential. (Author abstract) (48 Refs)

56. THE REGULATION OF THE HUMAN ANTITUMOR IMMUNE RESPONSE TO ORGAN-SPECIFIC NEOANTIGENS.

Thomson DM, Phelan K, Scanzano R, Fink A, Bortin MM, Ferrone S, Martin WJ, Pious D
Montreal General Hosp. Res. Inst., Montreal General Hosp., 1650 Cedar Ave., Mont real, Quebec H3G 1A4, Canada
Transplant Proc; 13(4):1952-1954 1981

The mechanism of WBC adherence inhibition (LAI) is discussed. By increasing free intracellular Ca, Ca ionophore and ouabain stimulate a positive response. Lidocaine affects the binding of Ca to membranes and inhibits Ca++ flux; without Ca++ flux, antigen binding does not mediate LAI. Substances that increase intracellular cyclic AMP do not alter an LAI-positive response but convert to positive LAI-negative responses of patients with large tumor burdens. When prostaglandin E2 synthesis is blocked by indomethacin, the response of LAI-positive WBC is unaffected. When the generation of metabolities of the lipoxygenase pathway is blocked, the LAI-positive response is negated. Bridging of cytophilic antibody receptors on membranes of monocytes by tumor antigens is probably transduced into information useful to the cell. The transduction is believed to involve the opening of Ca channels in the cell membrane. Metabolites of the lipoxygenase pathway play an important role in mediating the response to the tumor antigens. Control of the biochemical reaction activated by Ca entry seems to involve intracellular cyclic nucleotide levels. LAI in early cancers is discussed. (13 Refs)

57. ADHERENT IA+ MURINE TUMOR LINES WITH CHARACTERISTICS OF DENDRITIC CELLS. I. MORPHOLOGY, SURFACE PHENOTYPE, AND INDUCTION OF SYNGENEIC MIXED LYMPHOCYTE REACTIONS.

Cohen DA, Kaplan AM
Dept. Surgery, MCV/VCU Cancer Center, Medical Coll. Virginia, Virginia Commonwealth Univ., Richmond, VA, 23298
J Exp Med; 154(6):1881-1898 1981

Several adherent cell lines cloned from mouse P388 leukemia were isolated and characterized. The cell lines were morphologically and functionally similar to dendritic cells (DC) of the spleen with respect to dendritic processes, large numbers of mitochondria, surface expression of immune region-associated (Ia) antigens, and induction of syngeneic and allogeneic mixed lymphocyte reactions. Of adherent P388 cells, 24-69% were positive for Ia antigen, greater than 95% were positive for major histocompatibility D, splenic macrophage-associated, and T cell-associated antigens, and 0% were positive for surface immunoglobulins; positive values were obtained in 13-42%, greater than 95 %, 0, greater than 95%, and 0%, respectively, of nonadherent cells. Both adherent and nonadherent forms of P388 expressed Ia antigens, with a broad range of fluorescent intensity within each respective clone. Cell-to-cell variability of surface Ia antigens in both adherent and nonadherent cells was also detectable with antisera against I-A subregion antigens. Adherent cells strongly stimulated syngeneic and allogeneic T lymphocyte proliferation in the absence of antigen; cytotoxic T cells directed against the stimulators were induced only during allo geneic stimulation. Nonadherent P388

clones did not stimulate either allogeneic or syngeneic T cell proliferation. (28 Refs)

58. PERSISTENCE OF COLCHICINE RESISTANCE IN EHRLICH-LETTRE ASCITES TUMORS AND CELL STRAINS.

Granzow C
Inst. Cell and Tumor Biology, German Cancer Res. Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, W. Germany
J Cancer Res Clin Oncol; 102(1):57-69 1981

The effect of colchicine on mitoses of mutant HD33 Ehrlich-Lettre ascites cells growing in vivo and in vitro was studied. HD33 mouse ascites tumors are colchicine resistant. The LD50 of colchicine in mice bearing HD33 ascites tumors was 1.4 mg/kg body weight (bw), but a single dose of 3.33 mg colchicine/kg bw failed to suppress the anaphase of HD33 tumor mitoses of 24 hr. No change in the level of colchicine resistance was observed after 269 weekly transplantations of HD33 ascites tumors without colchicine. In suspension culture, growth of HD33 ascites cells ceased at 1.5 x 10(-6) M colchicine. 10(-5) M colchicine suppressed the anaphase of HD33 mitoses and produced typical C-mitoses within 1 hr. The same effects on mitoses of colchicine sensitive Ehrlich ascites cells in vitro were achieved with 10(-6) M colchicine. In HD33 ascites cell cultures grown without colchicine, only a slight increase in colchicine sensitivity was registered after 5 yr. Parallel cultures were propagated for the same period in the presence of 10(-7) M colchicine (HD33C ascites cells) without detectable growth alterations; the resistance level increased slightly. The limit of 10(-6) M colchicine was tolerated by the ascites cells in permanent culture without growth reduction (HD33CS ascites cells). Studies of 3H-colchicine binding suggest a permeability barrier of the plasma membrane as a mechanism of genetically fixed resistance. (Author abstract) (29 Refs)

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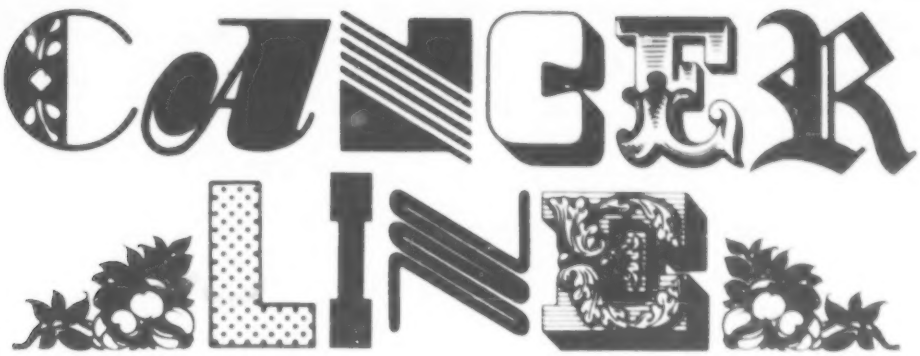

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. CHANGES IN CELL SURFACE GLYCOPROTEINS AND CARBOHYDRATE STRUCTURES DURING THE DEVELOPMENT AND DIFFERENTIATION OF HUMAN ERYTHROID CELLS.

Fukuda M, Fukuda MN
La Jolla Cancer Res. Foundation, La Jolla, CA, 92037
J Supramol Struct Cell Biochem; 17(4):313-324 1981

Studies of changes in cell-surface glycoproteins (GP) and carbohydrate structures during development and maturation of human erythroid cells (HEC) are reviewed. HEC were exam-

HE 20.3173/2:

ined by surface labeling with galactose oxidase/NaB[3H]4 (GO) or periodate/NaB[3H]4 (PI) techniques followed by digestion with endo-beta-galactosidase. Two classes of GP were detected: sialoglycoproteins lab eled by PI, and band 3 and band 4.5 GP labeled by GO. Overall results indicate the following: GP of bands 3 and 4.5 are barely detectable in proerythroblasts (ProE), become evident in late erythroblast (EB) maturation, and increase towards the mature RBC stage. Glycophorins are present in ProE before Hb synthesis and increase greatly in amount during maturation. GP with mol wt of 105,000 and 95,000 are major GP in ProE and decline during maturation, being minor GP in EB. Lactosaminoglycan increases during maturation from minimal amounts in ProE; the greatest increase occurs as bands 3 and 4.5 are expressed. Molecular branching of carbohydrate structures increases greatly during maturation. In general, maturation of HEC is marked by drastic changes in surface GP and carbohydrate structures. (45 Refs)

2. IDENTIFICATION OF EXPOSED SURFACE GLYCOPROTEINS IN UNDIFFERENTIATED AND DIFFERENTIATED MOUSE N-18 NEUROBLASTOMA CELLS.

Chen KY, Rinehart CA
Dept. Chemistry, Wright-Rieman Lab., Rutgers Univ., P.O. Box 939, Piscataway, NJ, 08854
Biochim Biophys Acta; 685(1):61-70 1982

A simple method is described that permitted rapid isolation of plasma membranes from mouse N-18 neuroblastoma cells. The purified plasma membranes gave a 10-fold increase in the specific activity of incorporated [3H]fucose over that of the cell homogenate. The specific activities of two other membrane markers, 5'-nucleotidase and alkaline phosphatase, increased 11-fold and 15-fold, respectively. Metabolic labeling with [3H]fucose identified a major fucosyl glycoprotein with apparent mol wt of 92 00. Three surface labeling methods together with SDS-polyacrylamide gel electrophoresis and fluorography were used to characterize and compare the surface glycoproteins of undifferentiated and differentiated N-18 cells. The galactose oxidase/NaB3H4 method labeled two major galactoproteins (Mr = 52,000, 42,000) in both undifferentiated and differentiated cells. The neuraminidase/galactose oxidase/NaB3H4 method revealed many sialylgalactoproteins. Among them, the 220-kdalton, 150-kdalton and 130-kdalton bands were at least 100% more prominently labeled in the differentiated calls whereas the 76-kdaltons and 72-kdalton bands were less prominently labeled in the differentiated cells when compared to their undifferentiated counterparts. The prominently iodinated protein bands in the undifferentiated cells had apparent mol wt of 130,000, 92,000, 76,000 and 72,000 as compared to 150-, 130-, 92-and 76-kdalton bands in the differentiated cells. The labeling data obtained will enable us to further study the changes of these identified surface glycoproteins, both quantitatively and topologically, during the differentiation of neuroblastoma cells. (Author abstract) (28 Refs)

3. THE ROLE OF GLYCOCONJUGATES IN METASTATIC MELANOMA BLOOD-BORNE ARREST AND CELL S URFACE PROPERTIES.

Irimura T, Nicolson GL
Dept. Tumor Biology, Univ. Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
J Supramol Struct Cell Biochem; 17(4):325-336 1981

The role of glycoconjugates in cell-surface and blood-borne implantation (BBI) properties of murine melanoma cells (MMC) were studied. MMC of lines B16-F1 (which has low BBI potential) and B16F10 (which has high BBI potential) were exposed in vitro to tunicamycin (0.5 ug/ml for 36 hr), which prevents glycosylation of glycoproteins by inhibiting formation of lipid-linked oligosaccharide precursors. Modification of cell-surface glycoproteins was examined by measuring the decrease in binding by sialogalactoproteins of 125I-labeled Ricinus communis agglutinin I. Treatment with tunicamycin reduced protein synthesis in B16-F10 cells by 50% and reduced DNA synthesis and growth rates in both MMC strains. Tunicamycin reduced BBI in the lungs of C57BL/6 mice receiving iv injections of MMC, without an apparent shift to extrapulmonary sites. Electron microscopic examination showed that B16-F1 and B16-F10 cells treated with tunicamycin developed morphologic changes, including rounding of the cells and formation of numerous surface blebs. Treatment with tunicamycin reduced in vitro adhesion of MMC to endothelial cell monolayers, endothelial basal lamina, and polyvinyl-immobilized fibronectin. Results suggest that tunicamycin reduces experimental metastasis of murine melanoma by modifying surface glycoproteins that determine the adhesive properties of MMC. (62 Refs)

4. NEURAMINIDASE ACTIVITY AND CELL SURFACE SIALIC ACID TURNOVER IN ROUS SARCOMA VIRUS TRANSFORMED CHICK EMBRYO FIBROBLASTS.

Myers-Robfogel MW, Spataro AC, Bosmann HB, Morgan HR
Dept. Pharmacology and Toxicology, Univ. Rochester Sch. Medicine and Dentistry, Rochester, NY, 14642
Cancer Biochem Biophys; 5(3):141-146 1981

Activities of neuraminidase (Ndase), cytidine monophosphate-N-acetyl neuraminic acid synthetase (CMP-AcNeu synthetase), and cytidine monophosphate-N-acetylneuraminic acid hydrolase (CMP-AcNeu hydrolase) were determined in primary cultures of chick embryo fibroblasts (CEF) transformed with the Schmidt-Ruppin strain of Rous sarcoma virus (RSV) and CEF infected with a Rous-associated virus that did not transform them. Total acid-labile sialic acid (SA) was decreased in RSV-CEF cells, but not in RAV-CEF cells. Ndase-accessible SA was increased above the control levels in both transformed and untransformed infected cells. Total Ndase activity in whole cell homogenates assay by neuraminlactitol-(3H) was elevated at both pH 4.3 and pH 6.8 in RSV-CEF but not in RAV-CEF, and cell-surface Ndase activity was also elevated in RSV-CEF according to release of (14C)-SA from cells preincubated with CMP-(14C)N-acetylneuraminic acid. Trans formed and untransformed cells did not differ from controls in activities of CMP-AcNeu synthetase and CMP-AcNeu hydrolase. Ndase activity in the cell and at the cell surface appears important in maintaining the altered SA-containing macromolecules of the transformed cell surface. (16 Refs)

5. A DIRECT EVIDENCE FOR THE EARLY MEMBRANE DESIALYLATION IN COBALT-IRRADIATED MOUSE LYMPHOCYTES.

Banchereau J, Dubos M, Agneray J, Drouet J
Laboratoire de Biochemie, ERA CNRS 396, Centre d'Etudes Pharmaceutiques, 92290 Chateney-Malabry, France
Biochem Biophys Res Commun; 104(2):512-516 1981

Spleen cells of Swiss C 17-DUPLAN mice exposed to 450 rads from 60Co irradiation exhibited a significant, 34% decrease in membrane sialic acid residues compared to controls, as shown by a periodate-tritiated borohydrate radiochromatography assay. A biphasic curve of desialylation in response to

increasing dosage of radiation suggests that the desialylation concerns either a subpopulation of spleen lymphocytes or a subpopulation of cell surface glycoconjugates. Several hypotheses to explain the decrease in membrane sialyl residues in irradiated cells are discussed. (20 Refs)

6. IMMUNOCHEMICAL STUDIES OF INFECTIOUS MONONUCLEOSIS. VIII. A GLYCOPROTEIN FROM SHEEP ERYTHROCYTES WITH SIALIC ACID-DEPENDENT RECEPTOR PROPERTIES.

Fletcher MA, Caldwell KE, Latif ZA, Cayer M, Claflin A
Dept. Medicine R42, Univ. Miami Sch. Medicine, P.O. Box 016960, Miami, FL, 33101
J Immunol; 128(2):976-982 1982

A glycoprotein was solubilized from sheep erythrocyte membranes with host aqueous ethanol. The glycoprotein was purified by phosphocellulose chromatography, ethanol precipitation, lipid solvent extraction, and DEAE chromatography. In water solution the glycoprotein existed as globular aggregates with a diameter of 7.1 +-2.2 nm. In the presence of sodium dodecyl sulfate, 80% of the material exhibited a subunit mol wt app of 27,000. Approx 10% of the material had a mol wt app of only 9,000 and another 10% had a mol wt of 35,000. All three fractions were reactive with Paul-Bunnell heterophile antibody from the sera of patients with infectious mononucleosis and with Limulus polyphemus lectin. These activities were destroyed by neuraminidase treatment. Complete inhibition of the rosetting of sheep red blood cells by 4 x 10(5) human peripheral blood lymphocytes was seen at 100 to 200 ug glycoprotein/ml. Neuraminidase-treated glycoprotein was not inhibitory. Pronase-derived sialoglycopeptide was inhibitory. Most likely, the receptor for lymphocytes resides in the carbohydrate portion of the glycoprotein. By using 125I-glycoprotein, binding studies were carried out that yielded an estimate of approx 2 x 10(5) binding sites for sheep erythrocyte glycoprotein per lymphocyte. Purified glycoprotein contained 44.4% amino acid. Carbohydrate components and th eir molar ratios were sialic acid (1.0):galactose (1.0):N-acetylglucosamine (1.3): N-acetylgalactosamine (1.2). (Author abstract) (33 Refs)

7. STIMULATION OF TYROSINE-SPECIFIC PHOSPHORYLATION BY PLATELET-DERIVED GROWTH FACTOR.

Ek B, Westermark B, Wasteson A, Heldin CH
Biomedical Center, Inst. of Medical and Physiological Chemistry, Uppsala Univ., Uppsala, Sweden
Nature; 295(5848):419-420 1982

The ability of platelet-derived growth factor (PDGF) binding to cellular receptors to stimulate kinase activity was studied in membranes prepared from cultured human foreskin fibroblasts or glial cells. PDGF stimulated the incorporation of 32P into membrane proteins of both fibroblasts and glial cells, promoting, in particular, the phosphorylations of a 175,000-molecular wt (Mr) and a 130,000-Mr protein, in comparison with the control incubation, in which no growth factor was added. Slight effects of PDGF were also seen in other components of the autoradiogram, associated with both increased and decreased phosphorylation. In the presence of epidermal growth factor (EGF), 32P incorporation increased preferentially in a 170,000-Mr protein of the fibroblast membrane preparation and in both a 170,000-and a 150,000-Mr component of the glial cell membrane preparation. The results indicated that different proteins serve as substrates for the kinases stimulated by the two factors. In membranes of the A431 epithelial cell line, only EGF appreciably increased phosphorylation. The specificity of the PDGF-stimulated kinase activity was studied in relation to the type of phospho-accepting amino residue involved. The mechanisms of action of EGF and PDGF are discussed. (34 Refs)

8. EFFECT OF RETINOIC ACID TREATMENT OF F9 EMBRYONAL CARCINOMA CELLS ON THE ACTIVITY AND DISTRIBUTION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE.

Plet A, Evain D, Anderson WB
Unite Institut National de la Sante et de la Recherche Medicale 188, 75014 Paris, France
J Biol Chem; 257(2):889-893 1982

Treatment of F9 teratocarcinoma cells with all trans-retinoic acid previously has been shown to induce differentiation to parietal endoderm and to enhance the responsiveness of these cells to the addition of cyclic AMP. Evidence is presented that retinoic acid treatment of F9 cells causes an increase in both cytosolic- and plasma membrane-associated cyclic AMP-dependent protein kinase activities. Retinoic acid elicits a progressive increase in cytosolic kinase activity through 72 hr. Cyclic AMP-dependent kinase activity of the plasma membrane fraction is enhanced within 3 hr of retinoid treatment of F9 cells and reaches a maximum at 15 hr after the addition of retinoic acid. The amount of regulatory subunits of cyclic AMP-dependent protein kinases I (RI) and II (RII) present in the subcellular fractions was quantitated by photoaffinity labeling with 8-azido-cyclic [32P]AMP. In the cytosol, the amount of RI and RII both increase with the period of retinoid treatment through 24 hr, and remain elevated through 5 days; the ratio of RI/RII (approx 2.5) found in the cytosol remains constant following retinoic acid treatment. RI is much more prevalent than RII (RI/RII = 7.5) in the plasma membrane prepared from untreated F9 cells. Retinoic acid treatment elicits a preferential increase in the amount of the RII regulatory subunit associated with the membrane fraction. After 15 hr of retinoid treatment, a 1.6-fold increase in RI is observed, while membrane-associated RII is increased 4.5-fold. After 24 hr of retinoic acid treatment, the level of RI returns toward the level noted with untreated cells, while the amount of RII remains significantly elevated through 5 days of retinoid treatment. These findings suggest that the increase in cyclic AMP-dependent protein kinase activity and the specific accumulation of RII associated with the plasma membrane fraction may be early events of retinoic acid action to mediate eventual cellular differentiation. (Author abstract) (38 Refs)

9. ALKALINE PHOSPHODIESTERASE I AND ALKALINE PHOSPHATASE I IN PLASMA MEMBRANES OF HERPES SIMPLEX VIRUS TYPE 1 TRANSFORMED HAMSTER CELLS.

Schneider D, Brauer D, Falke D
(c/o Falke) Inst. Medical Microbiology, Div. Experimental Virology, Johannes Gutenberg-Univ., Hochhaus Augustusplatz, D-6500 Mainz, W. Germany
Biochim Biophys Acta; 685(1):94-104 1982

Plasma membrane extracts from Herpes simplex virus Type 1 transformed hamster embryo fibroblasts were chromatographed on Lens culinaris lectin coupled to Sepharose (LcH-Sepharose) and analyzed by dodecyl sulfate polyacrylamide gel electrophoresis. Coomassie blue-staining revealed two major protein bands with apparent mol wt of 125,000 and of about 75,000-90,000. In plasma membranes isolated from these tumor cells prior labeled with [3H]fucose or [3H]glucosamine these

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bands contained the highest amounts of incorporated radioactivity. Separation by LcH-Sepharose-affinity chromatography as well as metabolic labeling clearly demonstrates their glycoprotein character. The 125,000 protein coincides with alkaline phosphodiesterase I activity with a Km of 6.10(-4) M for TMP p-nitrophenyl ester and is competitively inhibited by UDP-N-acetylglucosamine. This enzymatic activity is also present in normal hamster embryo fibroblasts. Gel electrophoresis of the Lens culinaris lectin-binding glycoproteins from plasma membranes of normal hamster embryo fibroblasts additionally revealed a strong alkaline phosphatase activity represented by an apparent mol wt of 150,000, while HSV1 hamster tumor cells contain only a very weak activity of this enzyme activity. HSV-lytically infected cells, however, have unchanged levels of alkaline phospha tase activity, whereas alkaline phosphodiesterase activity increases slightly. (Author abstract) (36 Refs)

10. RADIATION-INDUCED STRUCTURAL CHANGES IN HUMAN ERYTHROCYTE MEMBRANE PROTEINS REVEALED BY SODIUM DODECYL SULFATE/ POLYACRYLAMIDE GEL ELECTROPHORESIS.

Todo T, Yonei S, Kato M
Lab. Radiation Biology, Faculty of Science, Kyoto Univ., Kitashirakawa, Sakyo, Kyoto 606, Japan
Radiat Res; 89(2):408-419 1982

Differences in the radiosensitivity of the major proteins of human erythrocyte membranes was investigated. It was determined that disulfide formation is a minor event and more complex reactions are involved in the radiation-induced damage to membrane proteins. Analysis of the proteins by sodium dodecyl sulfate/polyacrylamide gel electrophoresis revealed the appearance of both cross-linked aggregates and radiolytic products of spectrin. Spectrin was the most sensitive of the erythrocyte membrane proteins to x-irradiation. (34 Refs)

11. LIPID COMPOSITION OF FRIEND LEUKEMIA CELLS FOLLOWING INDUCTION OF ERYTHROID DIFFERENTIATION BY DIMETHYL SULFOXIDE.

Rittmann LS, Jelsema CL, Schwartz EL, Tsiftsoglou AS, Sartorelli AC
(c/o Sartorelli) Dept. Pharmacology, Yale Univ. Sch. Medicine, Comprehensive Cancer Center, New Haven, CT, 06510
J Cell Physiol; 110(1):50-55 1982

The rates of incorporation of (14C)glycerol (14CG) and 3H-methyl choline (3HMC) into Friend erythroleukemia cells, both untreated and exposed to 1.5% dimethyl sulfoxide (DMSO), were determined. Both 14CG and 3HMC were incorporated linearly into lipids for up to 240 min; DMSO did not alter incorporation over the time period studied. Except for decreases in the proportion of sphingomyelin and diphosphatidylglycerol and a slight increase in phosphatidyl ethanolamine, phospholipids of cells differentiated by exposure to DMSO did not differ from control cells. The fatty-acid chain length did not appear to change substantially in differentiated cells, but a 15.6% decrease in saturation in the neutral lipid fraction and a 3% saturation decrease in the phospholipid fraction in DMSO-treated cells were noted. DMSO-induced cultures contained significantly less free and esterified cholesterol than did the individual control cells. The initial event involved in DMSO-induced differentiation of Friend leukemia cells apparently does not involve a change in phospholipid synthesis; that observed changes in membrane fluidity appear not to be due to changes in overall fatty-acid chain length in total neutral lipids and phospholipids. The effect of DMSO on cholesterol content indicates that maturation of the murine erythroleukemia cells is related to alterations in the cholesterol:phospholipid ratio. (48 Refs)

12. TRANSMEMBRANE ORIENTATION OF PALMITOYL-COA:LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE IN MICROSOMES ISOLATED FROM AN ALVEOLAR TYPE II CELL ADENOMA AND RAT LIVER.

Renooij W, Snyder F
(c/o Snyder) Medical and Health Sciences Div., Oak Ridge Associated Univ., P.O. Box 117, Oak Ridge, TN, 37830
Biochim Biophys Acta; 666(3):468-474 1981

The transverse localization of palmitoyl-CoA:lysophosphatidylcholine acyltransferase in the membrane of microsomal vesicles isolated from mouse lung adenomas and rat liver was studied by treating intact and deoxycholate-disrupted microsomes with trypsin and pronase. The latency of mannose-6-phosphatase was preserved during protease treatment, suggesting that membrane integrity was not affected. In adenoma microsomes 35-50% and in liver microsomes 35% of lysophosphatidylcholine acyltransferase activity is accessible to the reaction of the proteases. Our results suggest that at least a sizable portion of the active center of the enzyme that is responsible for remodeling phospholipids is embedded in the membrane interior. Since enzymes involved in de novo lipid synthesis are reported to be located at the cytoplasmic surface of the microsomal membrane, our results support the notion that in lipid metabolism distinct metabolic pools might exist at opposite sides of the microsomal membrane. (Author abstract) (39 Refs)

MORPHOLOGICAL STUDIES

13. VERSATILITY OF BIOTIN-LABELED LECTINS AND AVIDIN-BIOTIN-PEROXIDASE COMPLEX FOR LOCALIZATION OF CARBOHYDRATE IN TISSUE SECTIONS.

Hsu SM, Raine L
Dept. Pathology, Rhode Island Hosp., and Brown Univ., Providence, RI
J Histochem Cytochem; 30(2):157-161 1982

Carbohydrate residues in tissue can be localized by a very sensitive, specific, and simple technique, utilizing a biotin-labeled lectin followed by an avidin-biotin-peroxidase complex (ABC). Distribution of peanut receptors in benign and malignant tissues was found to be quite different. Normal colonic mucosa occasionally shows small dot staining in the supranuclear cytoplasm, while the tumor cells of colonic adenocarcinoma tend to show diffuse cytoplasmic or membranous staining. Normal breast ductal epithelium shows membranous staining at luminal borders, while medullary carcinoma of the breast shows distinct membranous and cytoplasmic staining. The use of this avidin-biotin-peroxidase complex technique may contribute to understanding the process of tumorigenesis as well as providing a sensitive method to determine early stages of malignant transformation. (Author abstract) (9 Refs)

14. ULTRASTRUCTURAL CHANGES IN SURFACE TOPOGRAPHY, GLYCOCALYX AND PLASMA MEMBRANE INTERIOR OF TUMOR CELLS DURING EXOCYTOSIS OF MUCUS.

Alroy J, Ucci AA, Roganovic D, Jacobs JB, Merk FB

Dept. Pathology, Tufts Univ. Sch. Medicine and Veterinary Medicine, Boston, MA, 02111

J Submicrosc Cytol; 14(1):171-177 1982

Plasma membrane changes, which occur during exocytosis, were studied by electron microscopy in six specimens of primary mucous-producing adenocarcinoma of human urinary bladder. The luminal surfaces of non-secretory neoplastic cells display numerous microvilli, which have a thick ruthenium red-positive glycocalyx. Neoplastic secretory cells have a smooth luminal surface with relatively few microvilli. The glycocalyces of these cells are thin, especially at locations where the plasmalemma and underlying mucous granule membrane are in close opposition. The fused membranes often bulge into the lumen forming distinct protuberances. In freeze-fracture replicas the protuberances appear devoid of 7-8 mm intramembrane particles. Our results suggest that there are differences between non-secretory and secretory neoplastic cells that are expressed in surface topography, glycocalyx and internal membrane structure. Secretion-induced changes observed in plasma membranes of the neoplastic cells closely resemble changes that are known to occur in the plasmalemma of normal secretory cells. (Author abstract) (18 Refs)

15. FINE STRUCTURE OF SEMINIFEROUS TUBULES FROM PRENATALLY IRRADIATED RATS.

Abreu I, David-Ferreira JF

(c/o David-Ferreira) Cell Biology, Gulbenkian Inst. Science, 2781 Oeiras Codex, Portugal

Cell Tissue Res; 222(1):143-152 1982

Ultrastructural changes in seminiferous tubules from Wistar rats irradiated prenatally on the 19th day of gestation were studied. Pregnant female rats received 1.50 Gray whole-body irradiation. Testes from 3-mo-old prenatally irradiated rats and non-irradiated rats were studied with light and electron microscopy. Seminiferous tubules of the irradiated rats were considerably smaller in diameter than those from normal rats (175 vs 326 micron, respectively). Tubules consisted mostly of elements of the lamina propria and Sertoli cells. The lumen was usually reduced in diameter and occluded by the cellular processes of the Sertoli cells. Numerous vacuoles of various sizes were seen in and between Sertoli cells. Lipid droplets were seen in many cells. The basal lamina was thickened and split into two or four layers. Ultrastructural changes were seen in the nuclei, cytoplasms and cell membranes of Sertoli cells. Two types of vacuoles were seen: larger ones near the basal zone of the seminiferous tubule wall, between adjacent Sertoli cells; and smaller ones mainly localized in the Sertoli cell apical zone that showed an irregular contour and a limiting membrane. The number of gap junctions appeared to be increased in the tubules of the irradiated rats and extensive 'desmosome-like' structures were often observed contiguous with the gap junctions. Anomalous ectoplasmic specializations were observed perpendicular to the Sertoli cell membrane. Numerous heterochromatin clumps were associated with the nuclear membrane. (28 Refs)

16. SCANNING ELECTRON MICROSCOPIC STUDY OF CHANGES IN THE SURFACES OF LYMPHOKINE-TRE ATED GUINEA PIG MACROPHAGES.

Onozaki K, Homma Y, Miura K, Hashimoto T

Dept. Microbiology, Inst. Basic Medical Sciences, Univ. Tsukuba, Ibaraki-ken 305, Japan

J Reticuloendothel Soc; 30(6):471-481 1981

The surface architecture of guinea pig peritoneal exudate macrophages treated with partially purified lymphokines and cultured for different time periods was examined by scanning electron microscopy. Lymphokine-treated macrophages exhibited remarkable cell surface changes including enlarged petal-like ruffles as early as 2 hr after treatment. The characteristic ruffles were found in both rounded and spreading macrophages, indicating that the changes were common structural alterations of lymphokine-stimulated macrophages. However, macrophages in the control preparation or in the medium alone formed a few such enlarged ruffles. The formation of the unusual ruffles continued for 24 hr but disappeared after about 48 hr. Subsequently, the macrophages had numerous contracted cell surface ruffles with apparently smooth faces, and, after 72 hr, their surface architecture further changed to form more rough ruffles with acute angles. Because both macrophage migration inhibitory factor and macrophage activation factor were contained in the lymphokine preparation, the formation of such petal-like ruffles appeared to be related to the phenomena of migration inhibition and activation of macrophages. (Author abstract) (24 Refs)

17. THE MORPHOLOGY OF BASEMENT MEMBRANE FORMATION.

Merker HJ, Barrach HJ

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Eur J Cell Biol; 26(1):111-120 1981

A transplantable, basement-membrane (BM)-producing murine tumor was investigated using immunofluorescence and electron microscopy. Collagen Type IV, fibronectin, laminin, and a BM-proteoglycan (BM-1) could be discriminated in the intercellular substance (ICS). The occurrence of these substances and the electron microscopic findings speak for a comparability of the ICS of the tumor with the BM or normal tissues. Hence, a model has become available to study the morphology of BM-formation. The BM-producing cells are characterized by numerous, often enlarged cavities of the rough endoplasmic reticulum (RER) and a large Golgi complex. The BM-material is released into the ICS via prosecretion and electron-dense secretion granules. The morphology of the extracellular BM-material depends upon the fixation technique. After addition of tannic acid, typical BM-lamellae are formed, which in most cases show a granular structure. This is due to shrinkage and attachment of glycoproteins to the collagen skeleton. After ruthenium red or after glutaraldehyde fixation, the extracellular matrix consists of homogeneously distributed filamentous material with only few granular structures. This situation corresponds to the small amount of acid proteoglycans in the BM. The present findings facilitate the evaluation of BM-formation in other cell types. (Author abstract) (51 Refs)

18. THE BASEMENT MEMBRANE IN THE CARCINOMA IN SITU OF THE CERVIX: A MORPHOLOGICAL STUDY.

Minh HN, Smadja A, Lecomte D, Orcel L
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Eur J Obstet Gynecol Reprod Biol; 12(6):373-383 1981

The prognostic significance of stroma-invasion potential of carcinoma in situ of the uterine cervix attributed to histologic alterations of the basement membrane was investigated. Sixty-eight cases of carcinoma in situ were examined histologically after punch biopsies. Forty-six cases of undifferentiated carcinoma in situ arising from the squamous-columnar junction were found. Light microscopy revealed an epithelium composed of small tumor cells with disappearance of epithelial stratification and no surface maturation or keratin formation. A continuous and clearly delineated basement membrane parallel to the basal layer was also present. Electron microscopy revealed immature cells of great uniformity with ovai nuclei and axis vertical to the basement membrane. Desmosomes were rate and imperfectly differentiated. Twenty-two cases of differentiated carcinoma involved the squamous epithelium of the portio. Epithelial differentiation and stratification were always significantly disturbed and surface keratin formation was sometimes evident. The basement membrane had a fibrillar structure due to irregular reticular fibers. Electron microscopy revealed polygonal shaped cells with individual pleomorphism sometimes evident. Desmosomes were less frequent than in normal squamous epithelium; their processes were shorter and the dense intercellular plaque was usually not evident. Study of the basement membrane is a useful diagnostic criterion for stroma-invasion potential in carcinoma in situ of the cervix. (20 Refs)

MEMBRANE RECEPTOR/ BINDING SITES

19. FUNCTION AND REGULATION OF METHIONINE(5)-ENKEPHALIN AND ITS RECEPTORS IN MURINE NEUROBLASTOMA CELLS.

Gilbert JA, Knodel EL, Stenstrom SD, Richelson E
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J Biol Chem; 257(3):1274-1281 1982

A homogeneous class of enkephalin receptors found in murine neuroblastoma clone N1E-115 has been confirmed using a centrifugation assay employing cellular membranes. In intact N1E-115 cells, synthetic methionine(5)-enkephalin inhibited prostaglandin E1-induced intracellular cyclic AMP formation in a naloxone-sensitive manner. Upon demonstrating intracellular methionine(5)-enkephalin immunocytochemically, analyses of crude N1E-115 extract were made by radioimmunoassay or opiate recept or binding assay following fractionation by molecular sieve chromatography and high pressure liquid chromatography on a mu-Bondapak C18 column. Extracted methionine(5)-enkephalin immunoreactive material behaved similarly to synthetic methionine(5)-enkephalin in these analyses. Growth curve studies of the N1E-115 cells indicated that the quantity of methionine(5)-enkephalin immunoreactive material synthesized per milligram of cellular protein and the max number of enkephalin receptor sites per milligram of membrane protein increased as the cells progressed from logarithmic to stationary phase, with no change in the apparent affinity of the enkephalin receptors for [3H]methionine(5)-enkephalin. These data suggest that adrenergic clone N1E-115 has functional methionine(5)-enkephalin membrane receptors, that this clone synthesizes methionine(5)-enkephalin, and that both the enkephalin receptor number and the content of stored methionine(5)-enkephalin are regulated with respect to cell division. (Author abstract) (40 Refs)

20. INSULIN RECEPTORS IN HUMAN CANCER.

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Br J Cancer; 44(6,.917-920 1981

A variety of human cancers were tested for specific receptors for insulin. Sixty-seven tissue samples (40 tumors, 24 fat, 3 muscle) were obtained during surgery were homogenized; the pellet contained receptors for prolactin and insulin. The insulin-binding assay was performed using porcine unlabeled and insulin labeled with 125I using chloramine-T. Specific binding was measured using Scatchard analysis. All specific binding values were adjusted to a membrane-protein concentration of 3.0 mg/ml; range of values found was 0.5-5.0 mg/ml. Significant insulin binding was detected in all tumor specimens except one breast-tumor sample which had a low yield of membrane protein of 1.5 mg/ml compared to an av value of 33.1 mg/ml. 125I-insulin binding to different types of tumor was compared with insulin binding to normal placenta, fat and muscle. Degradation of 125I-insulin by membranes from normal and tumor tissue was compared in one specimen of colon carcinoma. Little difference was detected in the rate of insulin degradation. Rate of degradation of labeled insulin also appeared similar in different types of tumor (colon, stomach, and breast). Affinity of binding decreased with increasing receptor occupancy. Apparently insulin receptors in normal cells are preserved during malignant transformation. However, other studies have shown that tumors can also synthesize ectopic hormone receptors. Results suggest that insulin binding is a common property of human cancer, and that insulin may help maintain or stimulate the growth of human tumors. (13 Refs)

21. PROTON NMR STUDY OF THE BINDING OF CONCANAVALIN A ON MYELOMA PLASMA MEMBRANES.

Tellier C, Curtet C, Poignant S, Godard A, Aubry J
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Biochem Biophys Res Commun; 104(1):113-120 1982

Lipid extracts were prepared from the plasma membranes of MF2S cells derived from the murine plasmocytoma MOPC 173, grown as ascites in BALB/c mice. Concanavalin A (Con A) was preincubated 30 min with two different purified membrane samples at 37 C in nuclear magnetic resonance (NMR) spectroscopy vessels and analyzed. One of the two membrane fractions had a modified NMR spectrum after Con A treatment. Con A:lipid binding interactions are discussed. (15 Refs)

22. THE SURFACE CHARACTER OF SEPARATED PROSTATIC CELLS AND CULTURED FIBROBLASTS OF PROSTATIC TISSUE AS DETERMINED BY CONCANAVALIN-A HEMADSORPTION.

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Dept. Urology, Erasmus Univ. Rotterdam, Dr. Molewaterplein 50, Rotterdam, The Netherlands
Prostate; 2(1):11-21 1981

The surface character of cultured fibroblasts from prostatic tumors by concanavalin A binding was investigated. Normal

prostate, benign prostatic hyperplasia (BHP), and prostatic carcinoma (PC) tissues were used. Con A-coated human type O RBC were used for the Con A reactivity assay of separated epithelial cells and cultured fibroblasts. Three PC specimens, six BHP specimens, and one normal prostate were examined for Con A-mediated hemadsorption (HAD) to their epithelial cell surface. All malignant epithelial cells showed higher reactivity than benign epithelial cells. Con A-mediated HAD was highest in permanent cell lines and lowest in benign prostatic epithelial cells, with that of PC cells intermediate between them. Con A reactivity of PC fibroblasts was higher than that of BHP fibroblasts. The use of Con A-mediated HAD is useful for distinguishing malignant epithelial cells of human prostate from nonmalignant cells, although whether the alteration of PC fibroblasts is the cause or the result of carcinogenic transformation of prostatic tissue is unknown. (20 Refs)

23. RECEPTORS FOR THE LECTINS WHEAT GERM. RICINUS COMMUNIS I AND SOYBEAN IN AMELOBLASTOMAS AND NORMAL ORAL MUCOSA.

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Dept. Oral Surgery, Royal Dental Coll., Universitetsparken 4, DK-2100 Copenhagen 0, Denmark
Acta Pathol Microbiol Scand [A]; 89(6):439-449 1981

The histological distribution of receptors for the lectins Wheat germ (WGA), Ricinus communis I (RCA I) and Soybean (SBA) was examined in ameloblastomas and normal oral mucosa from 12 patients. The study utilized fluorescein-conjugated WGA, RCA I and SBA. Cell-membrane bound receptors for these three lectins were demonstrated in the spinous cell layer of the normal oral mucosa. WGA and RCA I receptors were also located in the basal cell layer, whereas SBA receptors were not detectable there. Cell-m embrane bound WGA receptors were shown in the epithelial cells of the ameloblastomas. Titrations showed significant differences in staining reactivity related to the morphology of the peripheral epithelial cells of ameloblastomas. The distribution of RCA I and SBA receptors in the peripheral cells was also related to the morphology of these cells and was independent of the histological types of the tumors. It is suggested that the distribution of these receptors is related to cellular activities such as cell differentiation and cell migration in the tumor and therefore possibly reflects the biological behavior of the tumors. (Author abstract) (28 Refs)

24. RECEPTOR CLUSTERING ON A CELL SURFACE. III. THEORY OF RECEPTOR CROSS-LINKING BY MULTIVALENT LIGANDS: DESCRIPTION BY LIGAND STATES.

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Math Biosci; 53(1/2):1-39 1981

A mathematical model for receptor cross-linking by a multivalent ligand containing a given number of identical functional groups capable of binding to a surface receptor is presented. A class of ligands of this type consists of haptens bound to a carrier. The initial binding of such ligands to a cell is followed by further reactions on the surface, which cross-link receptors and lead to the formation of ligand-receptor clusters. The model accounts for the possibility that clustering can be competitively inhibited in the presence of monovalent haptens. The concept of an effective valence for the ligand (number of reactive groups capable of binding simultaneously to surface receptors) can account for steric hindrance and the inability of some ligand determinants to participate in cross-linking reactions due to their incorrect orientation. When the effective valence is greater than 2, receptor clustering can be measured by the following criteria: (1) the fraction of receptor sites occupied by multiply bound ligand, (2) the concentration of cross-links holding distinct receptors together, and (3) the concentration of cell surface receptors. These aggregation measures are dependent on ligand and monovalent hapten concentrations. Several theorems on the general shape and characteristics of cross-linking curves are confirmed. When a ligand can simultaneously bind two cell surfaces, the cross-linking curve is not symmetrical, although (as in the case of bivalent ligands) the curve generated using criteria (1) or (2) has one peak. The model does not account for intramolecular reactions and ring formation. The theory can break down at high degrees of cross-linking for receptors having two or more combining sites, in which case intramolecular reactions must be considered. (50 Refs)

TRANSPORT STUDIES

25. REDUCTION OF PROLINE TRANSPORT IN R3230AC MAMMARY CARCINOMAS BY ESTROGENS IN VIT RO.

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Biochem Pharmacol; 31(1):37-41 1982

The effects of estrogens on proline and leucine transport into enzymatically dissociated cells from the R3230AC mammary adenocarcinoma were studied. Estradiol-17beta demonstrated a time- and dose-dependent reduction of proline transport; at 10(-6) M, transport of proline was decreased by 50%. Kinetic analysis of these effects indicate that estradiol displayed characteristics of a noncompetitive inhibitor, with a Ki of 1.79 uM. Other estrogens, and the anti-estrogen tamoxifen, gave somewhat higher estimated Ki values and could be ranked as inhibitors as follows: estradiol-17beta greater than diethylstilbestrol greater than tamoxifen greater than estriol approx = estrone greater than estradiol-17 alpha. No effects of these estrogens on leucine transport were observed, indicating their selectiveness for the A system. The synthetic glucocorticoid, dexamethasone, was comparable to diethylstilbestrol as an inhibitor of proline transport, but dexamethasone also decreased transport of leucine. Testosterone and progesterone were approximately comparable to estrone in their actions. It is proposed that these actions of estrogens represent one potential mechanism whereby pharmacological levels exert therapeutic benefit in the treatment of advanced breast cancer. (Author abstract) (23 Refs)

26. CHOLINE UPTAKE IN CULTURED HUMAN Y79 RETINOBLASTOMA CELLS: EFFECT OF POLYUNSATURATED FATTY ACID COMPOSITIONAL MODIFICATIONS.

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J Neurochem; 38(3):650-656 1982

Choline uptake in Y79 human retinoblastoma cells occurs through two kinetically distinguishable processes. The high-affinity system shows little sodium or energy dependence, and it does not appear to be linked to acetyl-CoA:choline O-acetyltransferase. When the cells are grown in a culture medium

containing 10% fetal bovine serum, the high-affinity system has a K'm = 2.16 +-0.13 uM and V'max = 27.0 +-2.9 pmol/min/mg, whereas the low-affinity system has a K'm = 20.4 +-1.3 uM and V'max = 402 +-49 pmol/min/mg. Under these conditions, the polyunsaturated fatty acid content of the microsomal membrane fraction was increased by supplementing the culture medium with linolenic or docosahexaenoic acids (n-3 polyunsaturated fatty acids) or arachidonic acid (n-6 polyunsaturated fatty acid), the K'm of the high-affinity choline transport system was reduced by 40-60%. The V'max also was reduced by 20-40%. Supplementation with oleic acid, the most prevalent monounsaturated fatty acid, did not affect either kinetic parameter. The results suggest that one functional effect of the high unsaturated fatty acid content of neural cell membranes is to facilitate the capacity of the high-affinity choline uptake system to transport low concentrations of choline. This effect appears to be specific for polyunsaturated fatty acids but not for a single type, for it is produced by members of both the n-3 and n-6 classes of polyunsaturated fatty acids. (Author abstract) (34 Refs)

27. HEXOSE TRANSPORT IN UNDIFFERENTIATED AND DIFFERENTIATED BALB/C 3T3 PREADIPOSE CELLS: EFFECTS OF 12-O-TETRADECANOYLPHORBOL-13-ACETATE AND INSULIN.

O'Brien TG
Wistar Inst. Anatomy and Biology, Philadelphia, PA, 19104
J Cell Physiol; 110(1):63-71 1982

The hexose transport system of BALB/c 3T3 (clone A31T) cells, both before and after adipose conversion, was characterized and its modulation by 12-O-tetradecanoylphorbol-13-acetate (TPA) and insulin was studied by 14C-2-deoxyglucose (2DG) uptake and 3-O-3H-methylglucose (3MG) transport assay. TPA stimulated both 2DG uptake and 3MG transport systems in the undifferentiated cells, with the concentration stimulating half-maximal response being 3 nano (n) M for the 2DG system and 30 nM for the 3MG system. TPA also stimulated both systems in differentiated cells, but 3MG transport decayed much more rapidly than the level of 2DG upatke. Stimulation of 2DG uptake in undifferentiated cells by protein factors and TPA was as follows: TPA greater than or equal to fetal bovine serum greater than or equal to epidermal growth factor; insulin (170 nM) had no effect. Insulin did not stimulate 3MG transport in undifferentiated cells, but did activate it in differentiated cells over the range 1.7 nM -1.7 uM, with half-maximal response occurring at 0.17 uM; TPA was stimulatory in both types of cells, with half-maximal response at 30 nM in both. When the agents were used together insulin caused a significant additional stimulation of 3MG transport system of that caused by TPA alone. Cycloheximide treatment of differentiated cells concurrently with, or 6 hr prior to, insulin had no effect on its stimulation of 3MG transport, but did cause a 75% inhibition of the TPA-stimulated response. Long-term exposure of differentiated cells to insulin abolished the 3MG-transport acute stimulation response to insulin, but not the TPA acute response; long-term exposure to TPA did not adversely affect either TPA or insulin response in differentiated cells. Apparently TPA and insulin stimulate hexose transport in adipose cells by different mechanisms. (30 Refs)

28. THE ACTIONS OF INSULIN AS A HORMONAL FACTOR IN BREAST CANCER.

Hilf R, Siiteri PK, Shellabarger, KorenmanSG, Rosen, Nandi
Dept. Biochemistry and Univ. Rochester Cancer Center, Univ. Rochester, Rochester, NY, 14642
Banbury Rep; 8:317-337 1981

Evidence implicating insulin as one of the hormones involved in the growth of breast cancer in animals (insulin-dependent and insulin-responsive tumors) is presented. Some of insuline actions related to neoplastic growth are described (insulin receptors and their regulation, insulin and glucose transport, insulin and amino acid transport, and insulin as a regulator of estrogen receptors). Clinical data and the role of insulin in human breast cancer are reviewed. Direct effects of insulin are stimulation and inhibition of tumor growth. Indirect effects are regulation of estrogen receptors and antagonism of insulin effects by estrogen. (73 Refs)

29. MEMBRANE TRANSPORT PROPERTIES DIFFER FOLLOWING RETURN OF SERUM-DEPRIVED VERSUS CA++-DEPRIVED HUMAN FIBROBLASTS TO A PROLIFERATIVE STATE.

Tupper JT, Ryals WT, Bodine PV
Dept. Biology, Syracuse Univ., Syracuse, NY, 13210
J Cell Physiol; 110(1):29-34 1982

The unidirectional influxes of Rb+, an analog for K+ transport, and of 3-O-methylglucose, cycloleucine, and alpha-aminoisobutyric acid were monitored following stimulation of quiescent WI38 human lung fibroblast cells by Ca++ or fetal bovine serum. Quiescence in the cells had been induced earlier by deprivation of one of the same two stimulants. The absence of Ca++ at the time of serum stimulation inhibited the increase in transport usually observed upon serum stimulation, but Ca++ stimulation did not stimulate transport in Ca++-deprived cells yet still caused cells to progress to S phase. Ca++ and serum inhibition of cell division apparently have different mechanisms. (34 Refs)

30. EVIDENCE THAT A NA+/CA++ ANTIPORT SYSTEM REGULATES MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION.

Smith RL, Macara IG, Levenson R, Housman D, Cantley L
Dept. Biochemistry and Molecular Biology, Harvard Univ., Cambridge, MA, 02138
J Biol Chem; 257(2):773-780 1982

The Na+ and Ca++ transport properties of cultured murine erythroleukemia (MEL) cells have been investigated. We have previously shown that amiloride prevents dimethyl sulfoxide-induced MEL cell differentiation via inhibition of an essential Ca++ influx. Here we show that external Na+ inhibits Ca++ influx and stimulates Ca++ efflux from uninduced MEL cells. Increasing the internal Na+ concentration by a brief incubation of cells with ouabain stimulates the rate of 45Ca++ influx. Amiloride (40 uM) completely blocks the external Na+-stimulated 45Ca++ efflux and external Na+-inhibitable 45Ca++ influx. The same concentration of amiloride had no significant effect on net Na+ uptake. These results suggest that a significant fraction of Ca++ flux across the MEL cell plasma membrane occurs via a Na+/Ca++ antiport system and that amiloride prevents differentiation by blocking Ca++ influx through this system. The importance of a Na+/Ca++ antiport system for MEL cell differentiation is supported by the following observation: increasing the cellular Na+

level by a brief treatment with ouabain plus monensin accelerates MEL cell commitment as ef fectively as adding the Ca++ ionophore A23187. We suggest that dimethyl sulfoxide induces MEL cell differentiation by inhibiting the Na+ pump and consequently allowing Ca++ influx through the Na+/Ca++ antiport. (Author abstract) (37 Refs)

31. BIPHASIC UPTAKE OF IRON-TRANSFERRIN COMPLEX BY L1210 MURINE LEUKEMIA CELLS AND RAT RETICULOCYTES.

Takahashi K, Tavassoli M
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Biochim Biophys Acta; 685(1):6-12 1982

The kinetics of the cellular uptake of iron-transferrin complex was studied in L1210 murine leukemia cells and rat reticulocytes using 125I-transferrin. Saturation of transferrin with iron was necessary for optimal uptake. Following the incubation of cells with the radiolabeled complex a biphasic pattern of uptake was observed. The initial phase was rapid and relatively temperature-independent and was not altered by ethylamine, an inhibitor of transglutaminase activity which is necessary for receptor-mediated endocytosis. This phase was considered to result from receptor-ligand interaction which could be reversed to a great degree by replacement with unlabeled transferrin. A plateau was then reached, indicating a saturation of receptors. After 30 min a second phase of uptake was indicated by the second rise in the curve. This phase was slow, relatively temperature-dependent and could be abolished by ethylamine. It was interpreted as evidence of internalization of the ligand. Analysis of the data from competition studies with unlabeled transferrin indicated that the first phase might itself comprise a reversible and an irreversible step with a ratio of 5 to 1.4 for bound transferrin. Thus, the cellular uptake of iron-transferrin complex may consist of a reversible ligand-receptor interaction. Conformational changes may render this interaction irreversible and the internalization of the ligand may then follow. (Author abstract) (39 Refs)

32. STUDIES ON THE UPTAKE OF 67GA AND 59FE AND THE BINDING OF TRANSFERRIN BY CULTURED MOUSE TUMOUR CELLS.

Sephton RG, Harris AW
Cancer Inst, 481 Little Lonsdale St., Melbourne 3000, Australia
Int J Nucl Med Biol; 8(4):333-339 1981

Two cultured mouse tumor cell lines, HPC-108 (myeloma) and WEHI-22 (T lymphoma), were used in an investigation of transferrin promotion of 67Ga and 59Fe uptake by malignant cells. The effectiveness of added human transferrin could be readily demonstrated on the cells in culture medium containing fetal calf serum, but transferrin-promoted uptake varied considerably between different batches of the serum. In serum-free medium, transferrin at concentrations as low as 1 ug ml-1 promoted substantial uptake of 67Ga and maximal uptake of 59Fe. Saturation of the transferrin with iron pre-empted its activity toward both tracers, indicating their transport requires binding to the same sites on transferrin. High affinity receptors for transferrin on the tumor cells were characterized by measurement of the binding of diferric 125I-labeled human transferrin to whole cells at 15 C. The HPC-108 line possessed 4 x 10(5) receptor per cell, WEHI-22 1.5 x 10(5) per cell, the EMT6 sarcoma line only 5 x 10(4) per cell, all with a dissociation constant of 1.4-2.3 nM. Some aspects of 67Ga and 59Fe uptake were explained by the characteristics of this receptor and the previously demonstrated difference between the two tracers in affinity for transferrin. (Author abstract) (16 Refs)

33. THE ROLE OF TRANSFERRINS IN GALLIUM UPTAKE.

Larson SM, Grunbaum Z, Rasey JS
Nuclear Medicine Section, Medical Service (111), Veterans Admin. Medical Center, Seattle, WA, 98108
Int J Nucl Med Biol; 8(4):257-266 1981

In certain animal tumor systems, transferrin appears to play a key role in the cellular uptake of radiogallium. This process is mediated by a transferrin receptor which is present on tumor cells. In vitro studies using tumor cells growing in tissue culture demonstrate that the uptake is saturable so that the rate of 67gallium uptake is dependent on the degree to which 67Ga-transferrin competes with non-labeled transferrin for binding to tumor cell-associated transferrin receptors. Although the actual mechanism of this is not known, the evidence suggests that the 67Ga-transferrin complex is internalized into the cell, and that 67Ga is subsequently transferred to other subcellular macromolecules. Also, iron and gallium share a kinetically equivalent transport pathway. (Author abstract) (41 Refs)

34. INTRACELLULAR PHOSPHATE AND ITS POSSIBLE ROLE AS AN EXCHANGE ANION FOR ACTIVE TRANSPORT OF METHOTREXATE IN L1210 CELLS.

Henderson GB, Zevely EM
Dept. Biochemistry, Scripps Clinic and Res. Foundation, La Jolla, CA, 92037
Biochem Biophys Res Commun; 104(2):474-482 1981

Intracellular phosphate (Pi) transport in the absence of added Na+ was examined in mouse L1210 leukemia cells in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer. 33Pi uptake was compared in samples at 37 C and 4 C. A double reciprocal plot of the data yielded a transport kinetic constant of 1.7 milli(m)M for half-maximal transport; the maximal rate of transport was 50 picomoles/min/mg protein. Inhibition of 33Pi influx by increasing amounts of unlabeled Pi confirmed that 33Pi uptake was primarily carrier mediated. Methotrexate (MTX) also inhibited Pi uptake in the absence of added Na+. The Pi transport component affected by MTX represented approx 50% of the total uptake. Pi transport was partially inhibited by preincubation with carbodiimide-activated MTX, but the remaining 50% of activity was unaffected by subsequent addition of MTX. The Na+-dependent transport system of L1210 cells was inhibited by various anions in the order of effectiveness Pi greater than phyrophosphate greater than arsenate greater than sulfate greater than AMP = MTX. Pi evidently enters L1210 cells by at least two transport systems: a low-capacity Na+-dependent route that can be inhibited by MTX, and a high-capacity, MTX-insensitive system requiring Na+ that is comparable to the Pi transport systems in other mammalian cells. (20 Refs)

MEMBRANE BIOGENESIS AND METABOLISM

35. TWO DISTINCT INTRACELLULAR PATHWAYS TRANSPORT SECRETORY AND MEMBRANE GLYCOPROTEINS TO THE SURFACE OF PITUITARY TUMOR CELLS.

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Dept. Biochemistry and Biophysics, Univ. California, San Francisco, San Francisco, CA, 94143
Cell; 28(1):51-59 1982

The pituitary cell line, AtT-20, synthesizes adrenocorticotropic hormone (ACTH) as a glycoprotein precursor that is cleaved into mature hormones during packaging into secretory granules. The cells also produce an endogenous leukemia virus (MuLV) that is glycosylated after translation similar to the glycosylation of the ACTH precursor. Our evidence suggests that the envelope glycoprotein and some precursor ACTH get to the cell surface in a vesicle different from the mature ACTH secretory granule. Viral glycoproteins and ACTH precursor are released from the cells much sooner after synthesis than mature ACTH. Isolated secretory granules do not contain significant amounts of the envelope glycoprotein or ACTH precursor. Exposing cells to 8Br-cAMP stimulates release of mature ACTH four to five fold, but has little effect on the release of the ACTH precursor or the viral glycoproteins. We propose that the viral glycoproteins and some of the ACTH precursor are transported by a constitutive pathway, while mature ACTH is stored in secretory granules where its release is enhanced by stimulation. (Author abstract) (32 Refs)

36. ENHANCED SYNTHESIS OF BASEMENT MEMBRANE PROTEINS DURING THE DIFFERENTIATION OF RAT MAMMARY TUMOUR EPITHELIAL CELLS INTO MYOEPITHELIAL-LIKE CELLS IN VITRO.

Warburton MJ, Ferns SA, Rudland PS
Ludwig Inst. Cancer Res. (London Branch), Royal Marsden Hosp., Sutton, Surrey, SM2 5PX, England
Exp Cell Res; 137(2):373-380 1982

Rama 25, an epithelial cell line obtained from a dimethylbenzanthracene-induced rat mammary tumor differentiates spontaneously in culture forming elongated myoepithelial-like cells. The elongated cells form multilayered ridge structures from which cultures of elongated cells, relatively uncontaminated by epithelial cells, can be obtained. By using immunofluorescence techniques, both the elongated cells and the cells in ridges, but not undifferentiated Rama 25 cells, have been demonstrated to synthesize three basement membrane proteins, laminin, type IV collagen, and fibronectin. The identity of these base ment membrane proteins has been confirmed by immunoprecipitation. These proteins appear to be located in a fibrillar extracellular matrix. We suggest that the ability to synthesize basement membrane proteins by mammary epithelial cells in vitro on plastic is a characteristic of myoepithelial-like cells. (Author abstract) (20 Refs)

37. UPTAKE OF (3H)OUABAIN FROM THE CELL SURFACE INTO THE LYSOSOMAL COMPARTMENT OF HELA CELLS.

Cook JS, Tate EH, Shaffer C
Biology Div., Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
J Cell Physiol; 110(1):84-92 1982

Three cell fractionation approaches were used to show that (3H)ouabain (3HO) bound at sublethal concentrations to Na,K-ATPase on the surface of HeLa S3 cells is taken up at a rate corresponding to the enzyme turnover rate and is co-distributed with beta-hexosaminidase (BH) in a single particulate compartment within the cell. The compartment has the same sensitivity to digitonin, shear, and osmotic shock as the lysosomal marker BH, indicating that the 3HO is taken up in the lysosomal compartment. The so-called 'soluble' fraction of both BH and 3HO is shown to be an artifact of cell homogenization. The nonmetabolized 3HO is released over a period of 70 hr by an unknown mechanism, which is possibly exocytosis. (23 Refs)

38. TURNOVER OF PLASMA MEMBRANE PROTEINS AND GLYCOPROTEINS IN NORMAL AND REGENERATING LIVER AND MORRIS HEPATOMA 7777.

Tauber R, Reutter W
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Eur J Cell Biol; 26(1):35-42 1981

Insertion and loss of individual polypeptides and glycopolypeptides were investigated in the plasma membrane of hepatic tissue at three states of differentiation: (a) in normal liver, (b) regenerating liver and (c) in the Morris hepatoma 7777. Newly synthesized polypeptides and glycopolypeptides were inserted into the plasma membrane of all three tissues at different rates. In vivo labeling was performed with either L-[35S]methionine or L-[14C]fucose. Plasma membrane proteins were degraded heterogenously with at least two dynamically different classes in all three tissues. In normal liver three polypeptide bands, mol wt of 78 K, 94K and 165 K, were lost from the plasma membrane with an apparent half-life of less than 4 hr, whereas the bulk of plasma membrane proteins was degraded with an av half-life of 40 hr. In Morris hepatoma four polypeptide bands with mol wt of 22K, 36K 63K and 87K exhibited the same rapid degradation. Two of the short-lived polypeptides in liver (78K and 94K), and hepatoma (63K and 87K) were fucosylpolypeptides. There is evidence, that these two fucosylpolypeptides were lost from the plasma membrane by shedding into the cytosol. In normal liver rates of degradation of long-lived polypeptides correlated with the polypeptide mol wt, indicating that larger proteins may turn over at higher rates. In regenerating liver and hepatoma degradation of polypeptides of either high or low mol wt was inhibited to the same extent. It is concluded, that these readjustments in the relative rates of degradation of membrane polypeptides are a characteristic feature of proliferating hepatic tissue. (49 Refs)

39. REVERSIBLE AND IRREVERSIBLE STAGES IN THE TRANSITION OF CELL SURFACE MARKER DURING THE DIFFERENTIATION OF PLURIPOTENT TERATOCARCINOMA CELL INDUCED WITH RETINOIC ACID.

Ogiso Y, Kume A, Nishimune Y, Matsushiro A
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Exp Cell Res; 137(2):365-372 1982

Treatment of a pluripotent teratocarcinoma cell line with retinoic acid (RA) results in the disappearance of peanut agglutinin (PNA) receptors, accompanied with the decrease in F9 antigens and the enhanced secretion of plasminogen activator. However, this type of differentiation was inhibited by feeder cells. Furthermore, the transition of PNA receptor was reversible on the cells treated with RA for 2 days and became irreversible by an additional 2-day treatment with RA. Thus, two stages of teratocarcinoma cell differentiation - reversible and irreversible - were demonstrated. (Author abstract) (22 Refs)

40. EXPRESSION OF RED CELL SPECIFIC DETERMINANTS DURING DIFFERENTIATION IN THE K562 ERYTHROLEUKAEMIA CELL LINE.

Horton MA, Cedar SH, Edwards PA
Dept. Haematology, St. Bartholomew's Hosp., London, EC1A 7BE, England
Scand J Haematol; 27(4):231-240 1981

A human hemopoietic cell line (K562) which exhibits various erythroid characteristics, has been utilized as a model system for studying erythropoietic differentiation. We have analyzed the alterations in expression of cell membrane determinants which accompany the induction of Hb synthesis. We confirm that the K562 cell line exhibits a number of erythroid features: it expresses immunologically detectable membrane proteins, glycophorin A and spectrin, and can be induced, by addition of hemin or n-butyrate, to synthesize Hb. N and i-like blood group activities are demonstrable in uninduced K562, but band 3, ABH and other major alloantigens characteristic of mature erythrocytes are lacking. The acquisition of cell surface antigens typifying erythroid or other hemopoietic lineages has not been demonstrated following induction, although certain properties not associated with morphologically recognizable red cell precursors are lost. The relevance of these findings to the fetal nature and possible multipotentiality of the K562 cell line are discussed. (Author abstract) (25 Refs)

41. INCORPORATION OF 32P-PHOSPHATE INTO MEMBRANE PHOSPHOLIPIDS DURING INFECTION OF CULTURED HUMAN FIBROBLASTS BY HERPES SIMPLEX VIRUS TYPE 1.

Steinhart WL, Nicolet CM, Howland JL
Committee on Biochemistry, Bowdoin Coll., Brunswick, ME, 04011
Intervirology; 16(2):80-85 1981

Infection of cultured human skin fibroblasts by herpes simplex virus leads to increased incorporation of labeled inorganic phosphate into host membrane sphingomyelin. Incorporation into the other major membrane phospholipids, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine, was unaffected. Since sphingomyelin has been shown to serve as precursor to ceramides (via sphingomyelinase) in monkey kidney cells and since enhanced synthesis of ceramide-based glycolipids has been shown to occur after herpes simplex virus infection, we suggest that the observed increase in labeling of sphingomyelin may reflect mobilization for glycolipid synthesis. The distribution of phosphate label among the phospholipids of the viral envelope was identical to that among the phospholipids of the cellular cytoplasmic membrane fraction and differed from that of the nuclear fraction. (Author abstract) (14 Refs)

CELL ADHESION

42. A NON-COLLAGENOUS GLYCOPROTEIN FROM ELASTIC TISSUE ACTS AS SUBSTRATUM FOR GROWTH OF CELLS IN VITRO.

Knox P, Wells P, Serafini-Fracassini A
Dept. Biochemistry, St. George's Hosp. Medical Sch., Cranmer Terrace, London SW17 0RE, England
Nature; 295(5850):614-615 1982

In vivo, cells are in intimate contact with an extracellular matrix, one of the components of which is collagen; the adhesion of cells to collagen in vitro has been extensively studied. In several connective tissue matrices, collagen fibers are replaced to a variable extent by elastin fibers which, under the electron microscope, appear to be composed of an elastin core surrounded by a network of fibrils approx 11 nm in diameter, usually referred to as the microfibrillin component. These fibrils have been implicated, on morphological grounds, in the biosynthesis of elastin fibers. A glycoprotein referred to as structural glycoprotein (SCP), which has a mol wt (Mr) or 34,000 and aggregates to form cylindrical tactoids having a uniform diameter of 11 nm, has been isolated from bovine ligamentum nuchae. SGP was found to have amine oxidase activity, thus it seems to be functionally related to other peptidylysine oxidases involved in the biosynthesis of the elastin cross-links. Here we show that SGP acts as a substratum for fibroblasts and other cell types with fibronectin mediating the attachment and spreading of the cells. These results suggest that SGP may have a role in wound healing, morphogenetic movement and development of elastic tissues. (Author abstract) (16 Refs)

43. INVOLVEMENT OF FIBRONECTIN, VON WILLEBRAND FACTOR, AND FIBRINOGEN IN PLATELET INTERACTION WITH SOLID SUBSTRATA.

Lahav J, Hynes RO
Center Cancer Res., Massachusetts Inst. Technology, Cambridge, MA, 02139
J Supramol Struct Cell Biochem; 17(4):299-311 1981

The role of fibronectin (FN), von Willebrand factor (VWF), and fibrinogen (FG) in platelet function was studied. Platelets were obtained from healthy volunteers. Antiplatelet antiserum (aplt) and antiplasma FN antiserum (apFN) were raised by inoculation of rabbits. Aplt recognized pure plasma FN, FG, and a crude preparation of VWF, implying the presence of these factors either on the surface of or within platelets. An enzyme-linked immunosorbent assay detected no FN, VWF, or FG on the surface of unactivated platelets fixed shortly after gel filtration; results also indicated that unactivated platelets do not bind exogenous FN, VWF, or FG. Platelets that were allowed to bind and spread on either polystyrene or fibrillar collagen substrata expressed all three antigens, as detected by immunofluorescence methods. Treatment of spread and fixed platelets with Tween or Triton X-100 did not alter the amount of anti-FN bound by the platelets, indicating that all of the FN con-

tained by the platelets was expressed in exposed form on the surface. Activated platelets bound exogenous FN, VWF, and FG in a concentration-dependent manner. Spatial distribution of binding sites apparently were different for each of the three factors. Results indicate that activated platelets mobilize endogenous and exogenous FN, VWF, and FG, associating the factors with the platelet surface. Involvement of the factors in further binding properties of the platelets is suggested. (35 Refs)

44. DIFFERENCES IN HYALURONATE BINDING TO PLASMA AND CELL SURFACE FIBRONECTINS.

Laterra J, Culp LA
Dept. Microbiology, Case Western Reserve Univ. Sch. Medicine, Cleveland, OH, 44106
J Biol Chem; 257(2):719-726 1982

Human plasma-derived fibronectin (pFbn) and chick embryo fibroblast cell surface fibronectin (cFbn) were compared in a series of in vitro assays for binding to glycosaminoglycans (GAG) from various sources. Substratum-attached material, adhesive material that remains attached to serum-coated tissue culture substrata following ethylene glycol bis(beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid-mediated detachment of murine Balb/c 3T3 fibroblasts, was shown to be enriched, relative to whole cells, for highly N-sulfated heparan sulfate - the only GAG in these preparations that binds to Sepharose columns derivatized with either pFbn or cFbn. Adhesive material isolated from newly attaching and spreading fibroblasts contains 50% more of this heparan sulfate than material from long term substratum-attached cells. Both forms of fibronectin bound identically to preparations of 3H-heparin either under 'univalent' binding conditions, in which fibronectin or heparin were immobilized onto inert matrices of Sepharose or gelatin-Sepharose, or under the aggregating conditions of Yamada et al, in which fibronectin:GAG aggregates are trapped on Millipore membranes. Neither form of fibronectin bound metabolically radiolabeled high mol wt 3H-hyaluronate (HA) under the 'univalent' binding conditions. cFbn, but not pFbn, displayed appreciable affinity for hyaluronate only as a consequence of aggregation of the fibronectin. HA50, a 50-sugar oligomer of HA, was as active in binding cFbn as high mol wt HA, indicating that hyaluronate multivalency is not required for stable cFbn binding. These data are discussed with regard to the possible functional roles of cell surface heparan sulfate proteoglycans and hyaluronate binding to specific fibronectin classes in mediating physiologic adhesion and subsequent detachment of fibroblasts from the extracellular matrix. (Author abstract) (33 Refs)

45. CELL SURFACE MOLECULES INVOLVED IN FIBRONECTIN-MEDIATED ADHESION. A STUDY USING SPECIFIC ANTISERA.

Hughes RC, Butters TD, Aplin JD
Natl. Inst. Medical Res., The Ridgeway, Mill Hill, London NW7 1AA, England
Eur J Cell Biol; 26(1):198-207 1981

Attachment and spreading of baby hamster kidney (BHK) fibroblasts to fibronectin-coated surfaces can be inhibited by antibodies, and the (Fab)2 fragments, prepared against the cells. The antibodies reacted specifically with fibronectin-binding glycoproteins of the cell surface, the major components having mol wt of 130-140 K. The antibodies reacted also with mouse fibroblasts (L-cells) and Chinese hamster ovary (CHO)-cells and inhibited their fibronectin-mediated adhesion to inert surfaces. Antisera raised against material isolated from the underside of BHK cells spread out on fibronectin-coated substrata using a cleavable, photolabile heterobifunctional cross-linking reagent also affected the interactions of cells with fibronectin-coated surfaces. The antibodies stained the periphery of BHK cells and L-cells in a discontinuous periodic manner and immunoprecipitated as a major component a low mol wt (approximately 47 K) glycoprotein from cell extracts. These results indicate that different specific antisera modify cell-substratum adhesion, probably by interacting with different cell surface components. The properties of multiple factors involved in the attachment and spreading of cells to suitably adhesive substrata are discussed. (Author abstract) (31 Refs)

46. PRODUCTION AND DISTRIBUTION OF ENTACTIN AND GP-2 IN M1536-B3 CELLS.

Bender BL, Carlin B, Jaffe R, Temple T, Chung AE
Dept. Pathology, Presbyterian-Univ. Hosp., DeSoto at O'Hara St., Pittsburgh, PA, 15213
Exp Cell Res; 137(2):415-425 1982

The endodermally differentiated mouse embryonal carcinoma cell line M1536-B3 produces the basement membrane-associated glycoproteins entactin and GP-2 in cell culture. Immunological techniques coupled with light and electron microscopy were used to study the intracellular distribution and fate of these molecules. The two proteins were localized in cisternae of rough endoplasmic reticulum and in discrete membrane-bound vesicles. There was no evidence for their presence in the Golgi apparatus. The membrane-bound vesicles appeared to fuse with the plasma membrane and thereby transfer their contents to discrete foci on the cell surface. These foci became apparent as cell-to-cell contact was made and were prominent at cell-cell contact sites. They eventually coalesced into continuous densely stained extracellular bands of amorphous material. These bands formed a honeycomb of spaces lined by cells. It appeared that production of the extracellular matrix was probably stimulated by cell-cell contact, and that cell density rather than age of the cell cultures dictated the pattern of extracellular distribution. These results support the hypothesis that these molecules are involved in cell adhesion and multicellular organization. (Author abstract) (23 Refs)

47. METASTATIC TUMOR CELL ATTACHMENT AND INVASION ASSAY UTILIZING VASCULAR ENDOTHELIAL CELL MONOLAYERS.

Nicolson GL
Dept. Tumor Biology, Univ. Texas System Cancer Center, M. D. Anderson Hosp. and Tumor Inst., Houston, TX, 77030
J Histochem Cytochem; 30(3):214-220 1982

Two of the more important steps in blood-borne tumor metastasis are attachment of the circulating malignant cells to the vascular endothelium and subsequent extravasation or invasion out of the blood vessel. A model for this process has been developed using cultured monolayers of vascular endothelial cells that synthesize a basal lamina or extracellular matrix. We have used this model to study metastatic tumor cell-endothelial cell interactions such as attachment to endothelial cells and their subsequent retraction and exposure of endothelial basal lamina as well as the interactions of metastatic tumor cells with the basal lamina leading to invasion and solubilization of this extracellular matrix. Morphological, immunological, and enzymological analysis of these steps in the metastatic process can be obtained using the vascular endothelial cell monolayer model for attachment and invasion. (Author abstract) (50 Refs)

OTHER RELATED STUDIES

48. INTERFERON INHIBITS SENDAI VIRUS-INDUCED CELL FUSION: AN EFFECT ON CELL MEMBRANE FLUIDITY.

Chatterjee S, Cheung HC, Hunter E
Dept. Microbiology, Univ. Alabama, University Station, Birmingham, AL, 35294
Proc Natl Acad Sci USA; 79(3):835-839 1982

Interferon can affect several cellular functions, in addition to its antiviral activity. We report here that pretreatment of human cells with homologous interferon significantly inhibits cell fusion induced by Sendai virus and that this refractory state is accompanied by a decrease in cell plasma membrane fluidity. Multinucleate cell formation induced by beta-propiolactone-inactivated Sendai virus in human fibroblast cells (a system in which fusion results from an interaction of the viral glycoprotein with the cell membrane) was inhibited by more than 90% after addition of human interferon for 18-24 hr. This inhibition could be neutralized by antiserum to interferon. Furthermore, inhibitor studies with cycloheximide and actinomycin D clearly indicated that synthesis of protein and RNA is necessary to establish the resistant state. To determine whether the inhibition of Sendai virus-induced cell fusion resulted from interferon-induced changes at the cell plasma membrane, experiments were carried out using the fluorescence probe 1,6-diphenyl-1,3,5-hexatriene, which is capable of sensing molecular motions in the hydrocarbon core of the bilayer structure. A significant decrease in the membrane fluidity of interferon-treated cells was observed. It is likely, therefore, that the inhibitory effect on Sendai virus-induced cell fusion observed in interferon-treated cells results from an increased rigidity of the target cell membrane. (Author abstract) (31 Refs)

49. LOSS OF FIBRONECTIN AMONG THE SELECTIVE SURFACE PROTEIN CHANGES ASSOCIATED WITH P-FORMALDEHYDE PROMOTED MEMBRANE VESICULATION.

Rieber M, Rieber MS
Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas 1010-A, Venezuela
Biochem Biophys Res Commun; 104(2):844-850 1981

Radioiodination followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to detect proteins in vesicle fractions released when cultured cells were subjected to 25 milli(m)M p-formaldehyde and 2 mM dithiothreitol (DTT). Surface iodination of 'normal' WIRL-3 cells, 'transformed' R72/3 cells, normal rat kidney fibroblasts, and Rat-1 normal fibroblasts revealed upon electrophoresis a major 220 kilodalton (kd) fibronectin-like component. Vesiculation induced by the p-formaldehyde/DTT treatment decreased the component in all but the Rat-1 cells, and increased the lower mol wt fibronectin components, whether iodination took place before or after chemical vesiculation induction. In Rat-1 cells the vesiculation treatment caused a decrease in a surface-associated 140 kd component with an increase in a 130 kd component in the vesicular fraction. The release and selective degradation of cell surface proteins upon chemical vesiculation may involve surface protease activation. (12 Refs)

50. ACTION OF X-RAYS AND NEURAMINIDASE ON PINOCYTOTIC ACTIVITY AND MICROTUBULE-MICROFILAMENT SYSTEM OF EHRLICH ASCITES TUMOR CELLS IN MONOLAYER CULTURE.

Pfab R, Schachtschabel DO, Paul N, Kern HF, Hess F
Klinik und Poliklinik fur Strahlentherapie und Rontgendiagnostik, Univ. Marburg, Robert-Koch-Str. 8a, D-3550 Marburg, W. Germany
Strahlentherapie; 157(7):468-473 1981

Monolayer cultures of Ehrlich ascites tumor cells in exponential growth phase were treated with x-rays and neuraminidase alone or in combination. A radiation dose of 2 Gy (200 rad) and higher effected a significant inhibition of DNA synthesis and cell proliferation. Neuraminidase treatment in addition to irradiation did not modify the growth-inhibitory irradiation effect. Cells pretreated for 0.5 to 1.0 hr with neuraminidase (in Earle's salt solution) and cultured thereafter (4 hr) in serum-containing growth medium exhibited an enhanced development of the microtubule-microfilament-system. Morphometric analysis of microtubules on electron-microscopic sections revealed a nearly four-fold increased number in neuraminidase treated cells while the average length of microtubules was similar to controls. Pinocytosis as measured by the ultrastructural uptake of ferritin appeared to be increased following neuraminidase treatment. A connection between neuraminic acid containing components (glycoproteins or glycolipids?) of the cell membrane or cell surface, on the one hand, and the cytoskeleton and the endocytotic process of these tumor cells, on the other hand, is suggested. (Author abstract) (25 Refs) (In GER)

51. DECREASED UPTAKE AND RETENTION OF RHODAMINE 123 BY MITOCHONDRIA IN FELINE SARCOMA VIRUS-TRANSFORMED MINK CELLS.

Johnson LV, Summerhayes IC, Chen LB
Dept. Anatomy, Sch. Medicine, Univ. Southern California, 2025 Zonal Ave., Los Angeles, CA. 90033
Cell; 28(1):7-14 1982

A reduced uptake and retention of the mitochondria-specific membrane potential probe rhodamine 123 by feline sarcoma virus (FeSV)-transformed mink fibroblasts (64F3) has been detected. The decreased accumulation of rhodamine 123 by 64F3 mitochondria is not due to abnormal plasma membrane dye permeability, since after microinjection of the dye these cells are still unable to retain the dye at levels comparable to the untransformed parental cells, CCL 64. Nigericin, an ionophore that mediates an electrically neutral exchange of protons of potassium ions resulting in the elimination of the pH gradient across the mitochondrial membrane and a compensatory increase in mitochondrial membrane potential with continued respiration, increases both the dye uptake and the retention time in transformed 64F3 cells. These results suggest that mitochondria in FeSV-transformed mink cells may have an abnormally low mitochondrial membrane potential accompanied by a relatively high pH gradient. Since anionic metabolites such as pyruvate and glutamate are accumulated by mitochondria in proportion of the deltapH across the mitochondrial membrane, the abnormal mitochondria described here may contribute to the abnormal metabolic state of FeSV-transformed cells. (Author abstract) (26 Refs)

52. AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF MONOCLONAL ANTIBODIES TO CELL SURFACE ANTIGENS ON VIABLE CELLS.

Posner MR, Antoniou D, Griffin J, Schlossman SF, Lazarus H
Sidney Farber Cancer Inst., 44 Binney St., Boston, MA, 02115
J Immunol Methods; 48(1):23-31 1982

A rapid and inexpensive ELISA method is described which is suitable for the large scale screening of monoclonal antibodies to cell surface antigens. The use of acrylic plates and viable cells eliminates background and false positive reactions, and avoids modification of surface antigens caused by fixation. This facilitates easy and rapid detection of positives by visual inspection of the plates. The specificity and sensitivity of the method is comparable to indirect immunofluorescence or radioimmunoassay. The advantages of this ELISA system when compared to these methods and to previously described ELISA systems utilizing fixed cells are discussed. (Author abstract) (18 Refs)

53. CELLULAR INTERACTIONS IN THE CONTROL OF PROLIFERATION, MOVEMENT, AND ADHESION (ONCOLOGY OVERVIEW).

International Cancer Research Data Bank Program Office
NCI, Westwood Building, Room 10A18, Bethesda, MD, 20205
Cellular Interactions in the Control of Proliferation, Movement, and Adhesion (Oncology Overview) Steinberg, M, ed This Title is Available Through National Technical Information Service, Springfield, VA, as NTISUB/PB81-923005:; 76pp. 1981

ONCOLOGY OVERVIEWS are a service of the International Cancer Research Data Bank (ICRDB) Program of the National Cancer Institute. They are retrospective bibliographies containing 100-500 selected abstracts of published research on topics of high interest to cancer investigators. Included in the scope of this overview are several types of cell interactions which are impaired by neoplastic transformation. The abstracts cover the broad topics of cell proliferation, cell contact inhibition of locomotion, and cell adhesion. Within each of these areas specific subheadings deal with the effects of endogenous and exogenous agents on cell interaction, as well as the modification of cellular properties involved in the interactions between cells. There is also a section which contains theoretical models and general review articles.

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. HUMAN MELANOMA-ASSOCIATED ANTIGEN P97 IS STRUCTURALLY AND FUNCTIONALLY RELATED TO TRANSFERRIN.

Brown JP, Hewick RM, Hellstrom I, Hellstrom KE, Doolittle RF, Dreyer WJ
Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
Nature; 296(5853):171-173 1982

Cell surface glycoprotein p97 (mol wt 97,000), in SK-MEL 28 melanoma cells, was purified by affinity chromatography with monoclonal antibody, followed by sodium dodecyl-polyacrylamide gel electrophoresis. The N-terminal acid sequence was determined with the use of a new, highly sensitive protein sequencer. Amino acid sequence analysis of purified p97 yielded a sequence of 12 residues at the N-terminus. Computer

HE 20. 3173/2:

search of the Newat amino acid sequence library showed this sequence was homologous to the N-terminal sequence of transferrin; 7 of the 12 residues were identical. This homology is considered significant for two reasons: first, the two least common amino acid residues, Trp and Cys, are among those conserved, and second, six of the seven conserved residues are also conserved at the N-terminus of lactotransferrin. This homology was confirmed by the observation that antiserum to denatured p97 cross-reacted with denatured transferrin and lactotransferrin. p97 was also functionally related to transferrin and lactoferrin in that it binds iron. The presence of p97 on certain cell types suggests that its function may be translocation of iron. (15 Refs)

2. BIOCHEMICAL PROPERTIES OF THE HIGH-MOLECULAR-WEIGHT GLYCOPEPTIDES RELEASED FROM THE CELL SURFACE OF HUMAN TERATOCARCINOMA CELLS.

Muramatsu H, Muramatsu T, Avner P
Dept. Biochemistry, Kagoshima Univ. Sch. Medicine, Usukicho 1208-1, Kagoshima, Japan
Cancer Res; 42(5):1749-1752 1982

The present study deals with the biochemical properties of high-molecular-weight glycopeptides isolated from the surface of human teratocarcinoma cells. This cell surface material released by mild trypsin digestion from galactose-labeled human teratocarcinoma cells, Tera I and PA1, was digested extensively with pronase. Most of the resulting glycopeptides were large and were excluded from a Sephadex G-50 column. The properties of these large cell surface glycopeptides isolated from Tera I cells have been examined in detail. It is clear from these experiments that they are neither acidic mucopolysaccharides nor mucin-type glycopeptides with short oligosaccharide chains. Although the glycopeptides are hardly hydrolyzed by beta-galactosidase even after prior digestion with neuraminidase, around 30% of the glycopeptides are depolymerized by treatment with endo-beta-galactosidase from Escherichia freundii. The large cell surface glycopeptides from Tera I cells therefore appear to be very similar to the large glycopeptides seen on mouse embryonal carcinoma cells, which have core structures composed of galactose and N-acetylglucosamine. Like the mouse cell glycopeptides, a fractions of the large glycopeptides from these human cells bind to agarose-conjugated fucose-binding proteins and peanut agglutinin. (Author abstract) (14 Refs)

3. DIFFERENTIAL EFFECTS OF SODIUM BUTYRATE, DIMETHYL SULFOXIDE, AND RETINOIC ACID ON MEMBRANE-ASSOCIATED ANTIGEN, ENZYMES, AND GLYCOPROTEINS OF HUMAN RECTAL ADENOCARCINOMA CELLS.

Tsao D, Morita A, Bella A, Luu P, Kim YS
(c/o Kim) Gastrointestinal Res. Unit, Veterans Admin. Medical Center, 4150 Clement St., San Francisco, CA, 94121
Cancer Res; 42(3):1052-1058 1982

The effects of sodium butyrate, dimethyl sulfoxide (DMSO), and retinoic acid on the growth, morphology, carcinoembryonic antigen content, cell surface membrane-associated enzyme activities, and glycoprotein profiles of a human rectal adenocarcinoma cell line (HRT-18) in culture were compared. All three agents reversibly caused a marked increase in doubling times, a decrease in saturation densities, and a markedly reduced colony-forming efficiency in soft agar. Only butyrate caused gross morphological changes including cell enlargement, flattening, and increased membranous process formation. Carcinoembryonic antigen content was increased during culture in butyrate, while it was reduced by DMSO and unchanged by retinoic acid. The activities of membrane-associated enzymes were altered significantly in the butyrate-treated cells. For example, an increase in the activities of alkaline phosphatase (10-fold), gamma-glutamyl transpeptidase activity (3-fold) and sucrase activity (2-fold) was observed, while those of aminooligopeptidase and K+-stimulated phosphatase actually showed slight decreases. DMSO- or retinoic acid-treated cells showed a marked decrease in alkaline phosphatase activity, but other enzyme activities remained unchanged. Surface protein-labeling patterns of lactoperoxidase-catalyzed iodinated HRT-18 cells showed no significant change from the control cells following treatment with DMSO or retinoic acid. The most prominent change caused by butyrate treatment was the appearance of a major glycoprotein band with an apparent mol wt of 60,000. These data indicate that the use of butyrate, DMSO, and retinoic acid may provide useful information concerning the identification of differentiation-associated markers of human rectal cancer cells. Furthermore, these agents, although having similar effects on the growth properties, have different effects on the morphology and on the biochemical properties of human rectal cancer cells. (Author abstract) (48 Refs)

4. MODULATION OF PROTEIN PHOSPHORYLATION IN HUMAN COLON ADENOCARCINOMA CELL MEMBRANE PREPARATIONS BY EPIDERMAL GROWTH FACTOR IN VITRO.

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Int J Cancer; 29(3):277-282 1982

Incubation of membranes prepared from the human colon adenocarcinoma cell line LoVo in vitro with [gamma-32P]ATP demonstrated numerous components whose phosphorylation was stimulated several fold by epidermal growth factor (EGF). One major component of Mn 170 K, which was either undetectable or very weakly phosphorylated in the absence of EGF, was primarily affected by exposure to EGF. The phosphorylation of the 170 K Mr membrane component required the presence of Mn+; Mg+ was ineffective. Although the phosphorylation of many LoVo membrane components was significantly modified by cAMP or dibutyryl - cAMP, none of these cyclic nucleotides appeared to be involved in the phosphorylation of the 170 K membrane component in the presence or absence of EGF. The phosphorylation system of the LoVo membranes efficiently utilized [gamma-32P]GTP in both the basal and EGF-stimulated reactions. All the membrane components phosphorylated in the absence or presence of EGF, except a band comigrating with the tracking dye front, were digested by trypsin. The possible glycoprotein nature of the 170 K dalton phosphoprotein was indicated by the fact that the 170 K dalton band comigrated with a periodic acid-Schiff base-stained band. These findings suggest that one of the biochemical steps in the mechanism of action of EGF in LoVo cells is enhanced phosphorylation of several membrane proteins, especially that of a glycoprotein of Mr 170 K, by a membrane-bound cyclic nucleotide independent protein kinase. (Author abstract) (23 Refs)

5. CELL SURFACE MODULATION IN MAMMARY CANCER.

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The Handbook of Cancer Immunology: Volume 8- Tumor Antigens: Structure and Function. Waters H, ed. New York, STPM Garland Press, 418 pp., 1981.

Studies of the cell surface modulation which accompanies malignant transformation in mammary cancer are reviewed. Evidence for cell surface antigens (CSAs) of mouse and rat mammary tumors has not shown whether neoplastic transformation is associated with distinct tumor-specific antigens. Virus-induced mouse mammary tumors contain virus-specific antibodies, which are also present in normal mammary cells and nonmammary tissues. In rats with carcinogen-induced mammary tumors, the tumor loses an organ-specific antigen at the time of spontaneous dissemination. The experimental mammary tumors may also show gene derepression, which leads to the expression of abnormal differentiation antigens and/or fetal-type antigens. The tumors can acquire new CSAs not found in the tissue of origin and can acquire or lose CSAs already present in normal cells. Neoplastic transformation is a complex process, especially with regard to the cells which become neoplastic and the influence of host factors (genetic, hormonal, or immunologic). Interaction of these factors determines the malignant state. (283 Refs)

6. CHEMICAL AND IMMUNOLOGICAL CHARACTERIZATION OF RAT ASCITES HEPATOMA ALKALINE PHOSPHATASE: A COMPARISON WITH THE LIVER ENZYME.

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J Biochem (Tokyo); 91(1):201-210 1982

Alkaline phosphatase was purified from plasma membrane of rat ascites hepatoma AH-130 cells by chromatographies on DEAE-cellulose and Affi-Gel Blue and preparative polyacrylam ide gel electrophoresis. The yield of the purified enzyme, finally as the denatured subunits, was about three times better than that obtained previously The purified enzyme, a glycoprotein, was analyzed for amino acid and carbohydrate compositions. The composition of the carbohydrate moiety, which amounted to about 18% by wt, indicated that both N- and O-glycosidic sugar chains were contained in the protein. The purified alkaline phosphatase (as the denatured subunits) was used to produce monospecific antibody, which was found to have the same immunological reaction with the native antigen as the antibody raised against the partially purified native enzyme. Immunological analysis by Ouchterlony double gel diffusion and quantitative immunoprecipitation demonstrated that the hepatoma enzyme was identical with that of the liver. In standard polyacrylamide gel electrophoresis at pH 8.9, the hepatoma alkaline phosphatase migrated a little more slowly than the liver enzyme. Both enzymes, however, showed identical mobility after complete removal of sialic acid from them with neuraminidase. These results suggest that the only difference between the two enzymes was in the carbohydrate moiety, especially in the sialic acid content. (Author abstract) (35 Refs)

7. LESS MEMBRANE-ASSOCIATED ACTIN IN TRANSFORMED VERSUS NORMAL RAT KIDNEY CELLS.

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Cell Biol Int Rep; 6(2):177-187 1982

The amount of membrane-associated (MA) actin was compared in normal rat kidney (NRK) cells and their Kirsten viral transformants (442 cells). Entrapment of non-specific proteins and/or chemically induced alterations of plasma membrane incurred during isolation were overcome by employing an isolation procedure which does not require gradient purification, sonication, or the use of chemical membrane stabilizers or detergents. Plasma membranes were isolated by adhering NRK and 442 cells to a poly-L-lysine treated surface, and lysing them with hypotonic buffer. Results showed that MA actin to total MA protein ratio is significantly greater for NRK than for 442 cells. (24 Refs)

8. PERICELLULAR MATRIX AND CELL SURFACE GLYCOPROTEINS OF VIRUS-TRANSFORMED MOUSE EPITHELIAL CELLS.

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Cancer Res; 42(3):1147-1153 1982

Mouse embryo Mus musculus castaneous epithelial cells, transformed with Moloney murine sarcoma virus (MSV) or with ecotropic murine leukemia virus (MuLV), were analyzed for production of pericellular matrix glycoproteins. The nontransformed, MSV-transformed, and MuLV-transformed cells produced fibronectin, laminin, Type I collagen, and small amounts of Type III collagen when studied by immunofl uorescence using specific antibodies. The virus-transformed epithelial cells produced enhanced amounts of fibronectin into their growth media. Nontransformed M musculus castaneous epithelial cells mainly produced Type I collagen, as shown by metabolic labeling and polypeptide analysis. A significant increase in the glycoprotein production was seen by the MuLV-transformed cells, whereas small changes in the collagen production were apparent after MSV transformation. MuLV-transformed cells produced increased amounts of Type I collagen and also some collagenous polypeptides that comigrated with procollagen Type IV chains. The ratio of the procollagen Type I chains deposited in the matrix was altered in transformed cells. Radioactive surface labeling of the cells revealed changes of the high-mol-wt glycoproteins in both the MSV- and the MuLV-transformed cells. Unlike virus-transformed fibroblastic cells, these transformed epithelial cells deposited and retained connective tissue glycoproteins in their pericellular matrices. The results indicate that viral transformation modulates the pericellular matrix and surface glycoproteins of cultured mouse epithelial cells. The ability of virus-transformed epithelial cells to deposit pericellular matrices is a major difference between them and virus-transformed fibroblastic cells. (Author abstract) (46 Refs)

9. ALTERATIONS IN CELL SURFACE MEMBRANES IN CHINESE HAMSTER LUNG CELLS RESISTANT TO ADRIAMYCIN.

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Biochem Biophys Res Commun; 105(1):157-163 1982

An isolated culture of Chinese hamster lung cells (HT-1) exhibiting resistance to adriamycin was examined. Cell surface proteins of adriamycin-sensitive and -resistant cells were labeled with 131I using the lactoperoxidase catalyzed iodination technique. The iodination pattern of four different isolates of resistant cells was analyzed by polyacrylamide gel electrophoresis. Membrane proteins of resistant cells exhibited two alterations: a significant reduction in a major 100,000 mol wt protein and the presence of a 180,000 mol wt protein not found in sensitive cells. Additional studies using cells grown in the presence of [14C]glucosamine or [35S]methionine also revealed a membrane glycoprotein with a mol wt of 180,000 in adriamycin-resistant cells that was absent in sensitive cells. The fact that the four different drug resistant isolates all contained the same membrane changes suggests that cell surface alterations may play an important role in the resistant phenotype. The phenomenon might help to explain the adriamycin-resistance that certain patients acquire during adriamycin chemotherapy. (17 Refs)

10. MEMBRANE-BOUND SIALOGLYCOPROTEINS FROM RAT LIVER AFTER COMBINED EXPOSURE TO BENZO(A)PYRENE AND SULFUR DIOXIDE.

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Vopr Med Khim; 27(6):791-794 1981

The effect of carcinogens on metabolism of membrane-bound sialoglycoproteins (MBSGP) was studied in random-bred albino male rats. Animals were exposed to benzo(a)pyrene (BP) in combination with sulfur dioxide (SD). Within 2 wk, 6 wk, and 6 mo after exposure, rats were sacrificed and the distribution of MBSGP in the liver was determined. Combined exposure to BP and SD resulted in the increase of N-acetylneuraminic acid content in liver mitochondria and liver lysosomes, decrease of neuraminidase activity, and increase of beta-galactosidase activity. Carcinogens did not affect lipid peroxidation in the liver tissue. (20 Refs) (In RUS)

11. ASSAYS OF CELL SURFACE PROTEIN KINASE: IMPORTANCE OF SELECTING CYTOPHILIC SUBSTRATES.

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Eur J Cell Biol; 26(2):306-309 1982

The ability of phosvitin and histone to serve as substrates for possible protein kinase(s) at the cell surface was examined with regard to their suitability as indicators of such activity. While phosvitin did not interfere with the membrane barrier of HeLa cells, 3T3 and SV 3T3 cells, histone caused severe damage as indicated by both uptake of viability stains Trypan Blue and diamidino-phenylindol and by release of intracellular compounds such as lactate dehydrogenase, cAMP-dependent protein kinase(s), and metabolically prelabeled proteins. Where intactness of the cell membrane is prerequisite for verification of ecto-protein kinase, histone cannot be used as substrate. In contrast, we found that phosvitin is suitable for assays of cell surface located protein kinase activity. (Author abstract) (22 Refs)

12. CELL SURFACE GLYCOPROTEIN MARKERS FOR NEOPLASIA.

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Methods Cancer Res; 19:53-105 1982

Glycoproteins that serve as cell surface markers of neoplasia are described biochemically, in relation to viral transformation, and for various animal and human cells, and the methods employed are reviewed. The animal tumors and derived cell lines discussed include the following: neuroblastoma, melanoma, ascites, hepatoma, mammary carcinoma, lung carcinoma, and leukemias and lymphomas. Glycoprotein cell surface markers in human cells are reviewed in relation to melanoma, mammary carcinoma, lung carcinoma, colon carcinoma, ovarian carcinoma, and neuroblastoma. Metabolic labeling, isolation, purificatio n, mapping, and structural analysis techniques applied to glycoproteins are reviewed. Future studies in this field will probably take advantage of improved methods such as isoelectric focusing gels, lectin affinity chromatography, and monoclonal antibodies. Ultimately the metabolic and genetic origin of each macromolecule must be determined. In the meantime, glycoprotein cell surface marker studies may be expected to contribute to diagnostic and immunologic approaches to management of disease. (313 Refs)

13. COMPLEXITY OF THE HUMAN T LYMPHOCYTE-SPECIFIC CELL SURFACE ANTIGEN T3.

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J Immunol; 128(4):1560-1565 1982

A human T lymphocyte cell surface antigen T3 was found to be involved in several functions that were specific for the cells that express this marker on their surface. We show here that several glycoproteins were found in the immunoprecipitates prepared with a monoclonal reagent anti-T3. In addition to the major glycoprotein complex of 20 kd, glycoproteins of 25 to 28 kd, 37 kd, and 44 kd were detected in immunoprecipitates. The charge heterogeneity of the 20 kd and 25 to 28 kd proteins was caused by variable sialic acid content. The 20-kd T3 glycoprotein carried several 'complex-type' and endoglycosidase-H-sensitive sugar moieties. We found that the 20-kd protein could be labeled with 125I-iodonaphthylazide, a reagent that reacts with hydrophobic areas of proteins. (Author abstract) (24 Refs)

14. THE ROLE OF ANTIGEN AND ANTIBODY IN IDIOTYPE REGULATION AND TL MODULATION.

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The Handbook of Cancer Immunology: Volume 7- Immune Function and Dysfunction in Relation to Cancer. Waters H, ed. New York, Garland STPM Press, 372 pp., 1981.

Studies on the behavior of the thymus-leukemia (TL) antigen system, present on the membranes of leukemic cells and immature thymocytes of some mouse strains, showed several behavioral differences from the tumor-associated antigens (TAA) and the H-2 antigens. When TL-containing (TL+) cells were exposed to anti-TL antiserum, the expression of TL activity de-

clined and TL antigen was lost from the cell surface. This antigenic 'modulation' was reversible. It resembled the down 'regulation' of membrane hormone receptor activity in hormone-sensitive tumor cells converting to hormone resistance. When TL+ ASL-1 leukemia cells were exposed to sera containing both anti-TL and anti-TAA antibodies, TL activity declined but TAA activity remained unchanged. Antisera containing only anti-TAA antibody (in the presence of complement) did not lyse these tumor cells. The leukemia cells remained sensitive to anti-H-2 antisera ev en in the presence of H-2 antigen-antibody complexes on the surface membrane and the formation of antigen-antibody patches' and caps'. Anti-H-2 antiserum did not affect the metabolism of H-2 antigens. Antigenically distinct, unassociated membrane glycoproteins reacted independently to antisera containing multiple immunoglobulin specificities. These and other findings indicated that antigenic modulations are a much more complex event than an effect of an antigen-antibody complex on the cell surface. Somatic hybrids of TL+ and TL-negative cells formed TL antigens but did not undergo antigenic modulation, a further indication of the complexity of the cellular controls governing the effect of antiserum on 'antigen display'. Antigenic modulation may have been suppressed as a 'luxury' function after cell fusion. (67 Refs)

15. INHIBITION OF MURINE T CELL-MEDIATED CYTOLYSIS AND T CELL PROLIFERATION BY A RAT MONOCLONAL ANTIBODY IMMUNOPRECIPITATING TWO LYMPHOID CELL SURFACE POLYPEPTIDES OF 94,000 AND 180,000 MOLECULAR WEIGHT.

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Eur J Immunol; 12(1):60-69 1982

The monoclonal antibody methodology was used to identify membrane structures involved in T cell functions. To optimize chances to produce and detect relevant antibodies, a xenogeneic sensitization protocol was utilized and hybridoma supernatants were screened, on functional rather than structural grounds, for their ability to inhibit a given function. The test function was T cell-mediated cytolysis. Mouse cytolytic anti-allogeneic cell populations were used to sensitize a rat, the spleen cells of which were fused to produce hybridomas; the supernatants of the latter were screened for their ability to inhibit mouse T cell-mediated cytolysis in vitro. Several inhibitory antibodies were obtained, one of which, H35-89.9 monoclonal antibody, was studied in more detail. It inhibited specific and concanavalin A (Con A)-mediated cytolysis by T cells, by acting on the effector cells. it reversibly inhibited soluble antigen-, alloantigen and Con A-induced T cell proliferation (but not LPS-induced B cell proliferation), after the production of interleukin 2, by acting on the responder cells. It also had a desagglutinating effect on Con A and LPS blasts and on EL4 cells. It immunoprecipitated from thymocyte membrane preparations two structures of 94,000 and 180,000 apparent molecular wt, and recognized cell surface determinants on both T and B lymphocytes. Our findings suggest that several antibodies directed against distinct effector cell membrane structures inhibit cytolysis. The case of H35-89.9 monoclonal antibody, which exerts multiple functional effects and immunoprecipitates two membrane polypeptides, raises the problem of the various possible relationships between t hese structures and functions. (Author abstract) (48 Refs)

16. MONOCLONAL ANTIBODY TO TRANSFERRIN RECEPTOR BLOCKS TRANSFERRIN BINDING AND INHIBITS HUMAN TUMOR CELL GROWTH IN VITRO.

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Proc Natl Acad Sci USA; 79(4,part1):1175-1179 1982

A murine hybridoma has been obtained that produces a monoclonal antibody against the human transferrin receptor. In contrast to previously characterized monoclonal antibodies that recognize the transferrin receptor, this antibody, designated 42/6, blocks the binding of transferrin to its receptor and inhibits the growth of the human T leukemic cell line, CCRF-CEM, in vitro. Inhibition of cell growth was dose dependent, and as little as 2.5 ug of purified antibody per ml had a detectable effect, even though transferrin was present in the tissue culture medium in large molar excess. Cells grown in the presence of antibody for 7 days accumulated in S phase of the cell cycle. The addition of iron to antibody-treated cultures in the form of ferric complexes or ferrous sulfate did not overcome the growth inhibitory effects of the anti-transferrin-receptor antibodies. This result suggests that either transferrin is the only means by which CCRF-CEM leukemic cells can be provided with sufficient iron in vitro or that other factors in addition to iron starvation are involved in the antibody-mediated growth inhibition. The inhibition of cell growth by 42/6 monoclonal antibody suggests that monoclonal antibodies against proliferation-associated cell surface antigens, such as the transferrin receptor, may be useful pharmacological reagents to modify cell growth in vitro. (Author abstract) (46 Refs)

17. LEUKEMIC-LIKE MEMBRANE PROPERTIES ACQUIRED BY B LYMPHOCYTES WHEN DEPLETED OF 185,000-DALTON MACROMOLECULAR INSOLUBLE COLD GLOBULIN.

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Blood; 59(3):555-562 1982

Human peripheral blood lymphocytes can be phenotypically identified by the presence of one or both of two proteins, 225,000-dalton macromolecular insoluble cold globulin (225-MICG) and 185,000-dalton MICG (185-MICG). T cells synthesize and insert into their plasma membrane 225-MICG, null cells 185-MICG, and B cells both 225 and 185-MICG. In contrast, the monoclonal B cells of chronic lymphocytic leukemia are characterized by the presence of 225-MICG and the absence of 185-MICG. We have recently found it possible to chemically deplete 185-MICG from viable normal B cells by treating them with diisopropylfluorophosphate (DFP), thus making normal B cells phenotypically resemble leukemic cells. In the present report we determined whether certain peculiar properties of these leukemic cells would be associated with the normal B cells chemically depleted of 185-MICG. In normal B cells, SIg diffuses in the lipid bilayer to form clusters and caps under appropriate conditions, while in chronic lymphocytic leukemia (CLL) cells this does not occur. Normal B cells depleted of 185-MICG fail to undergo capping of SIg or surface MICG under appropriate conditions. Both DFP-treated B cells and CLL cells tend to rupture when smeared on a glass slide. Both CLL cells and DFP-treated B cells fail to secrete 225-MICG after it has been synthesized intracellularly. The relationship of these findings to the mechanisms of secretion and capping are discussed. (Author abstract) (42 Refs)

18. BIOCHEMICAL CHARACTERIZATION AND CELLULAR DISTRIBUTION OF A POLYMORPHIC, MURINE CELL-SURFACE GLYCOPROTEIN EXPRESSED ON LYMPHOID TISSUES.

Trowbridge IS, Lesley J, Schulte R, Hyman R, Trotter J

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Immunogenetics; 15(3):299-312 1982

A murine leukocyte surface glycoprotein (Mr = 95,000) has been defined by means of xenogeneic monoclonal antibodies. In normal hematopoietic tissues, the glycoprotein is found in highest amounts in the bone marrow. Flow cytometric analysis shows that essentially all bone-marrow cells express the glycoprotein and that it is a major component of a subpopulation of cells containing predominantly granulocytic precursors. In contrast, only about 5% of thymocytes express sufficient glycoprotein to be detected by flow cytometric analysis, although under stringent conditions up to 20% of thymocytes are susceptible to complement-mediated cytotoxicity using a monoclonal antibody against the glycoprotein. Functional assays showed that both prothymocytes and colony forming unit-spleen express the glycoprotein which is broadly distributed on murine hematopoietic tumor cell lines. However, although some Thy-1+ (T) cell lymphomas express large amounts of the glycoprotein, others do not express detectable quantities of the molecule. The glycoprotein is not restricted to hematopoietic cells and can be detected on lung, kidney, brain, and liver as well as cultured fibroblasts. Monoclonal antibodies against the glycoprotein cross react with an antigen present on human cells. As described in the accompanying paper, the glycoprotein exists in two antithetical allelic forms and we show that it is identical to a polymorphic surface molecule independently characterized by Colombatti and co-workers. (Author abstract) (41 Refs)

19. GENETIC CHARACTERIZATION OF A POLYMORPHIC MURINE CELL-SURFACE GLYCOPROTEIN.

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Dept. Cancer Biology, Salk Inst., Post Office Box 85800, San Diego, CA, 92138

Immunogenetics; 15(3):313-320 1982

As described in the preceding paper, monoclonal antibodies have been raised by i mmunization of rats with mouse hematopoietic cells which detect a major cell-surface glycoprotein (Mr = 95,000) of mouse bone-marrow cells of the granulocytic series. While most of the monoclonal antibodies detect this molecule on bone-marrow and spleen cells of all mouse strains, two antibodies recognize alternative allelic forms of the molecule. One alloantigen is expressed in all the remaining inbred strains examined. The alloantigens are codominantly expressed on the cells of F1 mice. Backcrosses of DBA/2 and C57BL/6 with F1 mice (B6D2F1) confirmed that a single genetic locus is involved in the expression of the two antigenic forms and demonstrated linkage to Ly-m11 which has previously been mapped to mouse chromosome 2. These genetic mapping experiments and the biochemical properties of the glycoprotein suggested that it might be identical to a glycoprotein first identified on murine fibroblasts by Hughes and August and designated Pgp-1. This has been firmly established by exchange of monoclonal antibody reagents and sequential immunoprecipitations. (Author abstract) (16 Refs)

20. TUBULIN AS A MAJOR CELL SURFACE PROTEIN IN HUMAN LYMPHOID CELLS OF LEUKEMIC ORIGIN.

Rubin RW, Quillne M, Corcoran JJ, Ganapathi R, Krishan A

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Cancer Res; 42(4):1384-1389 1982

Surface-exposed proteins of vinblastine-sensitive human lymphoid cell line of leukemic origin (CCRF-CEM) were examined by the lactoperoxidase-catalyzed iodination and two-dimensional polyacrylamide gel electrophoresis methods. Spots which comigrate with bovine brain tubulin and rabbit muscle actin were prominently labeled in the whole membrane but not in the high-speed supernatant fraction of the disrupted cells. Mild trypsinization of labeled cells removed the iodinated tubulin and actin without significantly affecting the protein staining pattern. Iodination of normal human lymphocytes resulted in no labeling of the tubulin or actin. The presence of surface-exposed tubulin in this leukemic cell line suggests a possible mechanism for their enhanced sensitivity to the cytotoxic action of vinblastine. (Author abstract) (28 Refs)

21. MACROPHAGE SURFACE COMPONENT GP160: SENSITIVITY TO PLASMIN AND OTHER PROTEASES.

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J Immunol; 128(4):1541-1544 1982

The correlation between activation of macrophages and increased secretion of plasminogen activator suggests that macrophages are exposed to the protease plasmin. Incubation of 125I-labeled, caseinate-elicited guinea pig peritoneal macrophages with plasmin cleaves surface protein, gp160, characterized previously by its sensitivity to trypsin. The gp160 fragments produced by plasmin (fr85 and fr71), which remain disulfide-bonded to the membrane, comigrate with the fragments produced by trypsin, indicating close or identical cleavage sites. No other detectable 125I-labeled surface component is cleaved by plasmin. Neither gp160 nor any other detectable 125I-labeled surface component was cleaved by a series of other proteases associated with inflammation including thrombin, collagenase, pancreatic elastase, leukocyte elastase, cathepsin G, and urokinase. Analysis with the use of homogeneous plasmin from guinea pig plasma shows that concentrations as low as 50 ug/ml cause measurable cleavage of gp160 in 30 min. (Author abstract) (23 Refs)

22. THE ASPARAGINE-LINKED SUGAR CHAINS OF PLASMA MEMBRANE GLYCOPROTEINS OF K-562 HUMAN LEUKAEMIC CELLS: A COMPARATIVE STUDY WITH HUMAN ERYTHROCYTES.

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J Biochem (Tokyo); 91(1):233-246 1982

The paper electrophoretic pattern of the oligosaccharides released from the plasma membranes of K-562 cells by hydrazinolysis was quite different from that of human erythrocyte membranes. Bio-Gel P-4 column chromatography in combination with sequential exoglycosidase digestion of the neutral oligosaccharide fractions revealed that all those from K-562 cells are of the high mannose type, while those from erythrocytes are of large complex type structures. Studies of the acidic oligosaccharides indicated that none of those obtained from K-

562 cells contained the beta-N-acetylglucosamine residue linked at the C-4 position of the beta-mannosyl residue of the trimannosyl core, which occurs in most of the asparagine-linked sugar chains of human erythrocytes. This indicates that the glucosaminyltransferase that forms the GlcNAcbeta1 to 4Manbeta1 to 4 group has not been expressed in K-562 cells. (Author abstract) (43 Refs)

23. ELECTROPHORETIC ANALYSIS OF RADIOLABELED CELL SURFACE PROTEINS AND GLYCOPROTEINS OF SOME HUMAN HEMOPOIETIC CELL LINES.

Chorvath B, Duraj J, Stockbauer P, Babusikova O
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Neoplasma; 28(6):625-632 1981

Cell surface proteins and glycoproteins of several human lymphoblastoid- and neoplastic hemopoietic cell lines were radiolabeled by lactoperoxidase catalyzed iodination and by sodium periodate/tritiated sodium borohydride. Electrophoretic patterns of radiolabeled proteins and glycoproteins obtained by electrophoresis under denaturing conditions (SDS-PAGE) were essentially similar for all examined lymphoblastoid cell lines, with a characteristic group of major radiolabeled glycoproteins gp44 and gp24,31. Characteristic, individually different and distinguishable patterns of radiolabeled proteins were observed in different neoplastic hemopoietic cell lines: T-leukemia cell line MOLT 3, erythroleukemic cell line K562, pre-B cell leukemia line NALM 6, and a promyelocytic leukemia cell line HL-60. Cell surface proteins of HL-60 promyelocytic leukemia cell line displayed some similarities to those of another myeloid leukemia cell line ML 3. Examined Burkitt lymphoma cell lines were similar to lymphoblastoid cell lines with some minor differences. Glycoprotein gp44, markedly labeled on lymphoblastoid cell lines, was absent on Burkitt lymphoma cell line Daudi. Electrophoretic patterns of cell surface proteins of blast cells from a few patients with leukemia examined simultaneously with the cell lines are described and discussed. (Author abstract) (28 Refs)

24. UNIQUE GLYCOPROTEIN-PROTEOGLYCAN COMPLEX DEFINED BY MONOCLONAL ANTIBODY ON HUMAN MELANOMA CELLS.

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Proc Natl Acad Sci USA; 79(4,part1):1245-1249 1982

A monoclonal antibody, 9.2.27, with a high specificity for human melanoma cell surfaces has been utilized for biosynthetic studies in M21 human melanoma cells to define a unique antigenic complex consisting of a 250-kilodalton N-linked glycoprotein and a high mol wt proteoglycan component larger than 400 kilodaltons. The 250-kilodalton glycoprotein has endoglycosidase H-sensitive precursors and shows a lower apparent mol wt after treatment with neuraminidase. The biosynthesis of the proteoglycan component is inhibited by exposure of M21 cells to the monovalent ionophore monensin; this component can be labeled biosynthetically with $^{35}SO_4$, is sensitive to beta-elimination in dilute base, and is degraded by both chondroitinase AC and ABC lyases, suggesting that it is a chondroitin sulfate proteoglycan. These data demonstrate that the antigenic determinant recognized by monoclonal antibody 9.2.27 is located on a glycoprotein-proteoglycan complex which may have unique implications for the interaction of glycoconjugates at the human melanoma tumor cell surface. (Author abstract) (29 Refs)

25. CHARACTERIZATION OF THE CONSTITUENT FATTY ACIDS IN THE PHOSPHOGLYCERIDES OF CHEMICALLY INDUCED POLYCYSTIC DISEASE AND GASTRIC TUMOR IN RAT.

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Dept. Biology, Kitasato Univ. Sch. Medicine, Sagamihara, Kanagawa 228, Japan
Dev Growth Differ; 23(5):507-513 1981

The positional distribution of fatty acids in choline phosphoglycerides and other phospholipids in hepatoma and other tumors is different from distribution in corresponding normal tissues; this difference may be due to changes in positional distribution of fatty acids in membrane phospholipids. One year old BN rats of both sexes received tap water containing 0.009% N-methyl-N'-nitro-N-nitrosoguanidine to induce polycystic disease or gastric tumors. After 10 mo, whole livers and whole stomachs of experimental and control rats were analyzed for phospholipid classes. In diseased rats, choline phosphoglyceride was about one half that of normals; sphingomyelin content was increased in diseased livers. With regard to composition of fatty acids at the 1-position, diseased livers showed an increase of approx 22% in 16:0 fatty acid and a decrease of approx 12% in 18:1 fatty acid. In diseased organs, fatty acids at the 2-position showed greater saturation. An increase in 15:0 fatty acid at the 2-position of ethanolamine phosphoglycerides was observed in diseased organs. (18 Refs)

26. EFFECTS OF DEFINED SYNTHETIC PHOSPHOLIPIDS ON GLYCOSYLATION PROCESSES. MODIFICATIONS OF MEMBRANE-BOUND N-ACETYLGLUCOSAMINYL-TRANSFERASE AND SOLUBILIZED SIALYL-TRANSFERASE ACTIVITIES.

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Chem Biol Interact; 38(3):275-285 1982

In this paper, we study effects of phosphatidylcholine synthetic derivatives on two different glycosyltransferasic systems, a membranous mitochondrial N-acetylglucosaminyl-transferase and a solubilized microsomal sialyl-transferase. From all our results, we proved the strong inhibitory effect of lysophosphatidylcholines in these glycosylation processes. We investigated then the relationship between this inhibitory effect and the chemical structure of the studied phospholipids. From this work, it became obvious that the most important inhibitory effect is obtained with a molecule containing a long aliphatic side chain in sn-1 of glycerol, a carbon atom devoid of any large group in sn-2 and an hydrophilic part in sn-3 (specially choline). (Author abstract) (19 Refs)

MORPHOLOGICAL STUDIES

27. DISTRIBUTION OF A 100K DALTON GLUCOSE-REGULATED CELL SURFACE PROTEIN IN MAMMALIAN CELL CULTURES AND SECTIONED TISSUES.

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Exp Cell Res; 138(1):63-72 1982

Previously, we described a glucose-regulated cell surface glycoprotein (100K-GRP) which is present on the surfaces of non-transformed human fibroblasts. The 100K-GRP is released into the culture media in a soluble form. In this report, we utilize an antibody raised to the 100K-GRP and indirect immunofluorescence to localize the 100 K-GRP on human and mouse cells in vitro, and on mouse tissues in vivo. On cultured cells, the 100K-GRP is associated with fibers in the pericellular matrix. Examination of sectioned mouse tissues reveals that the 100K-GRP is present in a wide variety of tissues. However, in contrast to fibronectin, it is not found in extracellular structures such as basement membranes. In vivo, the 1 00K-GRP is only found in close association with the surfaces of individual cells. (Author abstract) (20 Refs)

28. ULTRACYTOCHEMICAL LOCALIZATION OF ESTROGEN-STIMULATED GUANYLATE CYCLASE IN RAT UTERUS.

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Dept. Pathology, USUHS, Bethesda, MD, 20814
J Histochem Cytochem; 30(4):331-342 1982

Estrogens are known to increase cyclic guanosine monophosphate (cGMP) levels in the uterus of rats by enhancing guanylate cyclase (GC) activity. In the present study, the cytochemical localization of GC activity was studied in the uteri of immature and ovariectomized rats after treatment with diethylstilbestrol (DES), progesterone, estrogen antagonist (C1628), and a combination of DES and C1628. Twenty-four hr after the first dose of DES, moderate to strong guanylate cyclase activity was indicated by lead phosphate precipitate on the luminal microvillar and basolateral surfaces of epithelial cells, whereas strong activity was found on the plasma membranes of fibroblasts, endothelial cells, and myometrial cells. The enzyme activity in the epithelial cells declined slightly 24 hr after the second daily dose of DES. Uterine tissues from DES-treated rats that were preheated at 60 C for 30 min or preincubated with a GC inhibitor showed no reaction product. Guanylate cyclase activity was not observed cytochemically in the uterine tissues of the vehicle control (immature or ovariectomized) or progesterone- and C1628-treated animals. Weak guanylate cyclase activity was observed on the plasma membranes of epithelial cells and endothelial cells after doses of DES and C1628 were given simultaneously. The biochemical assays of the total homogenate in vitro indicated that uterine GC showed about a twofold increase after one dose of DES and a 1.3-fold increase following two doses (1 dose per day) of DES when compared with their respective nontreated controls, or with progesterone-treated uteri. GC was found in particulate (90%) and cytosol (10%) fractions. These data demonstrated that DES stimulated uterine guanylate cyclase activity, while progesterone and C1628 were ineffective at the doses used. Estrogen antagonist C1628 does not completely suppress the effect of DES. (Author abstract) (38 Refs)

29. INTRACELLULAR LOCALIZATION OF CERTAIN MEMBRANE GLYCOPROTEINS IN MOUSE T-LYMPHOMA CELLS USING IMMUNOFERRITIN STAINING OF ULTRATHIN FROZEN SECTIONS.

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J Cell Physiol; 110(2):203-212 1982

Morphology observed with frozen thin sections of mouse T-lymphoma cells was compared with that of epon thin sections, with special emphasis on membranous organelles. The intracellular localization of several types of membrane glycoproteins in the T-lymphoma cell system was examined. No significan t differences were found between epon and frozen thin sections. Immunoferritin staining on frozen thin sections was specific, reliable, and suitable for intracellular localization of various membrane glycoproteins based on studies using antibodies directed against Thy-1, a T-cell-specific membrane glycoprotein. Many of the membranous cellular structures of the T-lymphoma cells contained the concanavalin A-specific sugars, alpha-D-mannose and alpha-D-glucose. The intracellular distribution of the viral membrane protein, gp 69/71, was studied in lymphoma cells known to be productively infected with a C-type RNA virus. Gp 69/71 molecules were detected on the surface of free and budding C-type viral particles. Occasionally, gp 69/71 was also found in discontinuous patchlike arrays along certain regions of the T-lymphoma cell surface. Viral antigens were found in terminal regions of certain flattened rough endoplasmic reticulum membranes, in a diffuse pattern at certain regions of the stacked Golgi membranes, and in clustered arrays within small vesicle-like structures either close to the plasma membrane or at an apparent point of contact between an individual vesicle and the plasma membrane. (19 Refs)

30. THE DIFFERENTIAL ULTRASTRUCTURAL LOCALIZATION OF IMMUNOGLOBULIN HEAVY AND LIGHT CHAINS IN HUMAN HAEMATOPOIETIC CELL LINES.

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Br J Haematol; 50(3):445-459 1982

The ultrastructural localization of immunoglobulin heavy and light chains has been investigated in nine hematopoietic cell lines, using a technique which involves the treatment of lightly prefixed cells with saponin to allow penetration of the antibody-peroxidase conjugates. The synthesis and secretion of immunoglobulin was also studied in these cell lines. Immunoglobulin was found to be localized in the cisternae and on the membranes of the rough endoplasmic reticulum, perinuclear space and/or Golgi apparatus. In each case staining for heavy chains was weak or absent in the perinuclear space while staining for light chains was usually strong. Additionally in three cell lines immunoperoxidase staining indicated that heavy chains were absent from the Golgi apparatus despite the observed presence of light chains in the Golgi apparatus and the secretion of combined immunoglobulin into the supernatant. The results obtained suggest compartmentalization of the synthesis of light and heavy chains and indicate that the technique of immunoelectron microscopy may significantly contribute to an understanding of the mechanisms involved in immunoglobulin synthesis, intracellular transport and secretion. (Author abstract) (25 Refs)

31. ADRIAMYCIN INHIBITS THE FORMATION OF NON-BILAYER LIPID STRUCTURES IN CARDIOLIPIN-CONTAINING MODEL MEMBRANES.

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Biochim Biophys Acta; 685(2):137-143 1982

The effect of adriamycin on cardiolipin-containing model membrane systems have been studied by 31P-NMR, freeze-fracture electron microscopy and binding experiments. Adriamycin effectively inhibits the formation of non-bilayer lipid structures induced by Ca++ and cytochrome c in cardiolipin-containing liposomes. This drug also strongly inhibits the uptake of Ca++ by cardiolipin into an organic phase. These results are discussed in relation to the cardiotoxic effect of adriamycin and the possible importance of non-bilayer lipid structures for the functioning of the mitochondrion. (Author abstract) (25 Refs)

32. LOW TEMPERATURE-INDUCED DISPLACEMENT OF CHOLESTEROL AND INTRAMEMBRANE PARTICLES IN NUCLEAR MEMBRANES OF MOUSE LEUKEMIA CELLS.

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Cell Biol Int Rep; 6(2):137-145 1982

Low temperature-induced displacement of cholesterol and intramembrane particles (IMP) was studied in GRSL 18 cells from a spontaneous thymus-derived lymphoid leukemia in the GR/A mouse strain. To check the reversibility of changes induced by low temperature, cells equilibrated at 0 C were brought back to 37 C and equilibrated for 1 hr prior to treatment with glutaraldehyde (GA) containing 300 uM filipin (fil). When cells were equilibrated and fixed at 37 C, the distribution of IMP appeared to be random. The induction of particle-free areas was fully reversed by post-equilibration. When fixation with GA was followed by incubation with a mixture of fil and GA, cleaved surfaces of the plasma membrane showed numerous randomly distributed filipin-cholesterol (FC) complexes. After equilibration at 37 C, the FC complexes in the nuclear membrane were distributed as single complexes, or in small loosely packed groups, mixed with IMP. Cleaved membranes of the endoplasmic reticulum showed circles of FC complexes around IMP-free areas. In accordance with reversal of IMP-free areas, the change in distribution of FC complexes by chilling to 0 C was not done by post equilibration at 37 C; the complexes then again being spread singly or in small groups all over the cleaved surface. Fixation prior to chilling to 0 C inhibited the movement of cholesterol, resulting in a distribution of FC complexes similar to that at 37 C. Displacement of cholesterol was not inhibited by GA fixation when post-equilibration followed; most FC complexes were concentrated within the IMP-free areas. (18 Refs)

33. TISSUE CONNECTIONS IN A TRANSPLANTABLE VIRUS-PRODUCING SEBACEOUS ADENOMA OF THE MOUSE. II. A FREEZE-FRACTURE STUDY IN CONJUNCTION WITH FILIPIN.

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J Cancer Res Clin Oncol; 102(3):215-226 1982

The distribution, size, and configuration of intercellular junctions in the sebaceous tumors of mice were examined using the freeze-fracture technique. Three types of junctions were observed: desmosomes, gap, and tight junctions. Tight junctions in general consisted of short linear unbranched fibrils, and macular or complex tight junctional patterns were present only occasionally. Gap junctions ranged from small spots of 0.9 x 10(-3) um2 (um2) to areas of approx 0.46 um2. Desmosomes were the most frequent junctional specializations, and it is concluded that they are at least partially responsible for this tumor not being metastatic. Filipin in conjunction with freeze-fracture showed filipin-sterol complexes on the plasma membrane, the nuclear envelope, and the membranes of the endoplasmic reticulum and mitochondria. The intercellular junctions were devoid of these complexes indicating that these regions are low in cholesterol. (Author abstract) (33 Refs)

34. HISTOGENESIS OF STORIFORM TUMORS (ELECTRON MICROSCOPIC STUDY).

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Cesk Patol; 18(1):1-10 1982

The histogenesis of storiform tumors was studied by electron microscopic examinations of biopsy specimens from 29 skin histiocytomas, 10 sclerosing hemangiomas, 13 lenticular dermatofibromas and 10 protuberant dermatofibrosarcomas. Endothelia and pericytes with numerous transient formations, together with varying degrees of lipid or other accumulations and fiber production linked with the lateral membrane were features common to all tumors. The filamentous component of the tumor cells always had the properties of myofibrils with a tendency to aggregate in brush like formations dependent on the cell membrane which usually contained numerous pinocytotic vacuoles. Thus, it differed from the cells of storiform neurofibromas (4 cases), whose intracytoplasmic filaments showed no tendency to clustering or relation to the cell membrane. Moreover, no substantial collagen fiber production was observed in the finer lateral membrane. The results indicate that histiocytoma, sclerosing hemangioma and dermatofibroma belong to the same class of tumors consisting of mixed vasoformative tissues. Dermatofibrosarcoma is of the same but mostly of hemangiopericytic origin, providing that it is histogenetically uniform. Storiform neurofibroma is different from the above tumors structurally. (26 Refs) (In CZE)

35. ULTRASTRUCTURAL EVENTS IN THE TRANSLOCATION OF POLYMERIC IGA BY RAT HEPATOCYTES.

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J Immunol; 128(3):1181-1187 1982

It has been proposed that polymeric IgA is translocated from plasma to bile across hepatocytes of the rat liver by a secretory component-mediated, vesicular transport. To define the ultrastructural details of the proposed transport mechanism, we employed peroxidase-labeled antibody immunocytochemistry to localize secretory component in the rat liver and monitor the hepatic translocation of homologous myeloma polymeric IgA infused iv. Secretory component was found associated with the endoplasmic reticulum, Golgi complexes, cytoplasmic vesicles, and plasma membranes of the sinusoidal and canalicular surfaces of hepatocytes; secretory component at the sinusoidal surface was most prominent in micropinocytic invaginations or pits. Livers were examined for the sites of polymeric

IgA 5, 15, and 30 min after infusion. Evidence was obtained that polymeric IgA is translocated across hepatocytes by a series of events: (1) polymeric IgA binds selectively to secretory component on the external surface of the sinusoidal plasma membrane; (2) secretory component-IgA complexes are internalized in endocytic vesicles; (3) the vesicles migrate through the cytoplasm without association with lysosomes or Golgi complexes; (4) the vesicles fuse with the cytoplasmic surface of the bile canalicular membrane, where secretory component-IgA complexes are released into bile by exocytosis. (Author abstract) (47 Refs)

MEMBRANE RECEPTOR/ BINDING SITES

36. RESOLUTION OF HIGH AND LOW AFFINITY EPIDERMAL GROWTH FACTOR RECEPTORS. INHIBITION OF HIGH AFFINITY COMPONENT BY LOW TEMPERATURE, CYCLOHEXIMIDE, AND PHORBOL ESTERS.

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J Biol Chem; 257(6):3053-3060 1982

Receptors for the mitogen epidermal growth factor (EGF) have been implicated as mediators of cellular transformation and tumor promotion. 12-O-Tetradecanoylphorbol-13-myristate (PMA) causes immediate but transient reductions in the ability of human KB cells to internalize EGF by decreasing the affinity of cell surface EGF receptors 7-to 12-fold. After 90 min of PMA exposure, complete recovery of the high affinity apparatus occurs. Evidence is presented that the decreased binding capacity to 125I-EGF is caused by delaying the appearance at the plasma membrane of cryptic receptor sites of high affinity. Exposure of these high affinity binding sites is temperature dependent and is blocked by pretreatment of cells with inhibitors of de novo protein synthesis. After short incubation periods (0 to 60 min) with 125I-EGF, up to 90% of the internalized 125I-EGF can be rapidly dissociated from cells by the addition of P MA. This treatment kinetically reveals a trypsin-insensitive, nondissociating component of cell-associated 125I-EGF. With PMA, both the rate (maximal at 4 hr with PMA as compared to 24 hr) and extent (8-fold greater with PMA) of transfer of 125I-EGF into this trypsin-stable compartment is enhanced and accounts for the severalfold stimulation in 125I-EGF accumulation. This evidence suggests that the internalization of 125I-EGF can be resolved into two components, one into which EGF immediately partitions and is freely exchangeable with the extracellular medium and that is disrupted by PMA. A second and previously unknown compartment retains 125I-EGF in the presence of PMA after a short delay to a considerably greater extent than that of untreated cells. Thus, without PMA, EGF is internalized into this nondissociating intracellular compartment, but the transfer is only 5-10% as efficient. These results suggest that the accumulation of stable intracellular mitogen-receptor complexes may be directed by a population of very high affinity receptors which may be an element involved in the control of cellular growth and tumor promotion. (Author abstract) (46 Refs)

37. SPECIFIC HIGH AFFINITY BINDING SITES FOR SOMATOSTATIN-28 ON PANCREATIC BETA-CELLS: DIFFERENCES WITH BRAIN SOMATOSTATIN RECEPTORS.

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Endocrinology; 110(3):1049-1051 1982

Saturable and high affinity binding sites have been obtained for an iodinated somatostatin-28 (SS-28) analog, [Leu8,D-Trp22,125I-Tyr25] SS-28, in a membrane preparation from hamster insulinoma, mainly composed of pancreatic beta-cells. Specific binding is maximal after 1 hr incubation at 22 C and represents 65% of the total binding. KD for [Leu8,D-Trp22,Tyr25] SS-28 is 0.25 nM with the number of sites corresponding to 68 fmol/mg protein. The Kd for SS-28 (1 nM) is more than 5 times lower than that for SS-14. SS-28 analogs, such as [D-Trp22] SS-28 and analogs which selectively inhibit insulin release in vivo (Des-Asn5[D-Trp8,D-Ser13] SS-14 and Des-Asn19[D-Trp22,D-Ser27] SS-28), are the most potent compounds in this assay. C-terminal replacement of L-cysteine by D-cysteine reduces the apparent affinity of SS analogs. Unrelated peptides and the inactive analog Des-Trp8-SS-14 have no affinity for insulinoma binding sites. There are differences between the insulinoma SS binding sites and those monitored under similar conditions from rat cerebral cortex. In cerebral cortex, SS-14 and SS-28 have similar affinity for the binding sites, and the insulin selective analogs are less potent than SS-14. It is concluded that pancreatic beta-cells, as well as brain, possess high affinity binding sites for SS, but that they differ in some of their pharmacological properties. (Author abstract) (16 Refs)

38. INTERFERON-INDUCED ANTIVIRAL ACTIONS IN FRIEND LEUKEMIA CELLS: ROLE OF MEMBRANE GANGLIOSIDES.

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Virology; 117(2):391-400 1982

The interactions between interferon (IFN) and membrane gangliosides have been analyzed in Friend leukemia cells (FLC). The GM1-cholera toxin (CT) model has been selected as a reference system for a high-affinity ganglioside-ligand binding. Two gangliosides have been detected in FLC extracts: a disialoganglioside (approx 95%) and a monosialoganglioside (approx 5%) which migrate respectively as GD1a and GM1. Unilammellar liposomes containing either mixed commercial gangliosides or GD1a or GM1 bind three to eight fold more IFN (both partially purified and pure) than gangliosides-free liposomes. Only 3-4% of the input IFN was bound, whereas GM1-containing liposomes bound almost 50% of [3H]acetyl-CT. Neuraminidase treatment of FLC, which converts GD1a-like into the GM1-like ganglioside, does not significantly modify the IFN-induced antiviral effects. CT binding to FLC as well as CT-induced elevation of endocellular cyclic AMP levels in FLC were instead markedly increased by the same neuraminidase treatment. Further, pretreatment of FLC with either GD1a or GM1 or gangliosides extracted from FLC did not change the IFN-induced establishment of the antiviral state. These data provide convincing evidence that FLC gangliosides do not represent the IFN high-affinity receptors nor specifically mediate IFN effects, even though they bind IFN to some extent. (Author abstract) (26 Refs)

39. DOWN-REGULATION OF INSULIN RECEPTORS IN PRIMARY CULTURES OF R3230 AC RAT MAMMARY ADENOCARCINOMA CELLS.

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Endocrinology; 110(4):1155-1163 1982

Binding of insulin and Concanavalin A to primary cell cultures of the R3230AC rat mammary adenocarcinoma was studied as a function of time in culture. As the culture became confluent, the amount of insulin binding per cell increased with culture time and reached a plateau, whereas the binding of Con A to surface glycoproteins decreased to 50% of the initial value. Exposure of confluent cultures to insulin at 37 C resulted in down-regulation of the cell surface insulin receptors. The decrease in insulin binding was related to the ambient insulin concentration and the decreased numbers of receptors per cell with no apparent alteration in their affinity. The maximal decrease in receptor number was 60-70%. Cell cultures degraded significant amounts of insulin at 37 C, but the addition of bacitracin to the culture medium decreased the amount of degradation and increased the extent of down-regulation at each insulin concentration. Porcine proinsulin was less effective than insulin in competing with 125I-labeled insulin and inducing receptor down-regulation. Down-regulation of insulin receptors did not require protein synthesis. The rate of insulin-induced receptor loss was much faster than the decrease in insulin binding due to inhibition of protein synthesis by cycloheximide. The estimated half-life of the insulin receptor was 10.5 hr. Down-regulation of insulin receptors was reversible; regeneration of receptors to 50% of control levels occurred approx 9.6 hr after the removal of insulin and required protein synthesis. The results indicate that these mammary tumor cells retain the ability to regulate their insulin receptors. (Author abstract) (53 Refs)

40. DESENSITIZATION OF TUMOUR LEYDIG CELLS BY LUTROPIN: EVIDENCE FOR UNCOUPLING OF THE LUTROPIN RECEPTOR FROM THE GUANINE NUCLEOTIDE-BINDING PROTEIN.

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Biochem J; 202(3):739-745 1982

Purified rat Leydig tumors cells were pretreated with lutropin and the effect on the subsequent response to lutropin was determined. Maximal cyclic AMP production was achieved with the same concentration of lutropin in control and lutropin-pretreated cells; however, the maximum stimulated level in pretreated cells was only 30% of controls. The sensitivity to lutropin was decreased in lutropin-pretreated cells, as was the rate of maximal cyclic AMP production. However, cholera-toxin-stimulated cyclic AMP production was not decreased by lutropin pretreatment, and a potentiation was seen at all time points studied. Pre-incubation with lutropin caused a decrease in specific 125I-labeled human choriogonadotropin binding; however, this decrease was abolished if the cells were washed under acidic conditions, indicating that occupation but not loss of the lutropin receptors had taken place. THe effect of pretreating the cells with lutropin on adenylate cyclase activity in purified plasma membranes was also investigated. In plasma membranes from control cells both guanosine 5'-[beta-gamma-imido]triphosphate [p(NH)ppG] plus lutropin and NaF plus lutropin caused a 50-60-fold linear increase in cyclic AMP production over 40 min compared with 15-fold with p(NH)ppG and 6-fold with lutropin alone. In plasma membranes isolated from lutropin-treated cells the NaF-plus-lutropin- and the p(NH)ppG-stimulated cyclic AMP production rates were unchanged but no effect of lutropin could be demonstrated with or without added p(NH)ppG. In contrast the plasma membranes from dibutyryl cyclic AMP-treated cells had similar cyclic AMP production rates to control cells with all stimulants studied. The present evidence obtained from studies both with intact cells and with isolated plasma membranes indicates that the initial lutropin-induced desensitization of the rat Leydig tumor cell is due to a lesion in the hormone-receptor coupling to the guanine nucleotide regulatory protein. This process is apparently not regulated by cyclic AMP. (33 Refs)

41. RECEPTOR-ASSOCIATED CHANGES OF THE CATECHOLAMINE-SENSITIVE ADENYLATE CYCLASE IN GLIOMA CELLS DOUBLY TRANSFORMED WITH MOLONEY SARCOMA VIRUS.

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J Cell Physiol; 110(2):107-113 1982

Activity of adenylate cyclase (AC) and the receptor binding assay of 3H-dihydroalprenolol (DHA) for the beta-adrenergic receptor of C6 glioma cells doubly transformed with Moloney sarcoma virus (C6V-1 cells) were carried out as a model system for viral modification of the plasma membrane and compared with those of C6 cells. Intracellular cyclic AMP levels and intracellular phosphodiesterase, mitochondrial monoamine oxidase, and cytosomal enolase activities were measured in both cell lines. C6V-1 cells differed from C6 cells morphologically. C6V-1 cells were composed of large numbers of both round and fusiform cells, whereas C6 cells contained predominantly fusiform cells. C6V-1 cells grew faster than C6 cells with mean doubling times of 17 and 22 hr, respectively. The basal activity of AC was always lower in C6V-1 cells than in C6. Effects of Mg + +, prostaglandin E1, isoproterenol, and norepinephrine on AC activity were determined in both cell lines. Fewer DHA binding sites were observed in C6V-1 cells than in C6 cells. Intracellular cyclic AMP content in C6 and C6V-1 cells was 10.1 and 4.4 picomoles/mg protein, respectively. Phosphodiesterase activity in C6 cells was 3x greater than that in C6V-1 cells, with mean activities of 91 and 31 picomoles/mg protein, respectively. Both cell types showed type A mitochondrial monoamine oxidase activity in the range of approx 70-90 picomoles/min/mg protein. Enolase activity was higher in C6V-1 cells than in C6 cells, with specific activities of 1.6 and 2.3 units/mg protein, respectively. (30 Refs)

42. A SOLID-PHASE ASSAY OF SOLUBILIZED HELA CELL MEMBRANE RECEPTORS FOR BINDING GROUP B COXSACKIEVIRUSES AND POLIOVIRUSES.

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Virology; 118(1):148-156 1982

HeLa cell membranes were solubilized in sodium deoxycholate and adsorbed onto polystyrene microtiter plates to permit the measurement of receptors for binding group B Coxsackieviruses and polioviruses. Receptor activity was determined by measuring the amount of infectious virus attached to coated wells at 6 C. Conditions for optimal binding of virus were determined. Receptor binding activity for group B Coxsackieviruses was detected from as few as 2.5 x 10(4) cell equivalents/ml to provide a method which was over 400x more sensitive than the virus eclipse assay used previously. The assay re-

vealed a recovery of up to 7% of the receptor activity of whole cells based on virus saturation of receptors. The specificity of the binding activity of the immobilized receptors was demonstrated by virus attachment competition, as done previously for receptors on live HeLa cells. Application of this method to measure receptors in deoxycholate-solubilized fetal mouse brain revealed a 2,500-fold increase in sensitivity from that obtained in a virus-binding assay used previously. (Author abstract) (28 Refs)

43. INTERACTIONS BETWEEN LYMPHOCYTE MEMBRANE MOLECULES. II. CHARACTERIZATION OF AN INTERACTION BETWEEN B LYMPHOCYTE SURFACE IGD AND FC IGG RECEPTORS THAT DIFFERS FROM THE SURFACE IGM-FC IGG RECEPTOR INTERACTION.

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J Immunol; 128(3):1271-1277 1982

The B lymphocyte surface membrane receptors IgD (sIgD) and Fc IgG receptors (FcgammaR) were evaluated for interactions by means of immunofluorescence. Ligand-(F(ab')2 anti-delta) induced capping of SIgD resulted in co-capping of FcgammaR if the latter were occupied during the capping process by soluble antigen-antibody complexes (which themselves provided insufficient cross-linking to result in capping), but not if the FcgammaR were occupied by monomeric IgG or unoccupied. Capping of FcgammaR by highly cross-linked complexes did not cause co-capping of SIgD occupied by monomeric F(ab') anti-delta. The interaction between sIgD and FcgammaR was specific in that cross-reactions between ligands were excluded and ligand-induced capping of sIgD did not cause co-capping of ligand-occupied sIgM or I-A antigens. The sigD-FcgammaR interaction occurred on only approx 60% of B lymphocytes, and this B cell subpopulation did not correlate with other B cell subpopulations (CBA/N strain B cells and B cells bearing either large or small amounts of sIgD). The sIgD-FcgammaR interaction differed from the sIgM-FcgammaR interaction in that co-redistribution of the latter two receptors was induced by F(ab')2 anti-mu when the FcgammaR was occupied by monomeric IgG and involved nearly all B lymphocytes. The qualitative and quantitative differences between the sIgD-FcgammaR and sIgM-FcgammaR interactions suggest a mechanism whereby the two antigen receptors could provide different signals to the B lymphocyte. (Author abstract) (54 Refs)

44. LIGAND-INDUCED ASSOCIATION OF SURFACE IMMUNOGLOBULIN WITH THE DETERGENT-INSOLUBLE CYTOSKELETAL MATRIX OF THE B LYMPHOCYTE.

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J Immunol; 128(3):1198-1204 1982

Ligand binding is believed to induce surface immunoglobulin (Ig) to form a physical association with the underlying cell cytoskeleton. We investigated this interaction by use of nonionic detergents, which are known to dissolve membrane proteins but preserve a detergent-insoluble cytoskeletal residue. In the absence of ligand treatment, surface Ig in the B cell plasma membrane was fully dissolved by nonionic detergent; however, interaction with a ligand converted the receptor to a novel, detergent-insoluble state. The conversion of surface Ig to a detergent-insoluble form required receptor crosslinking but occurred independently of capping. Several types of experiments demonstrated that this form of Ig was not due to the size insolubility of immune complexes and involved a stable, noncovalent association of the receptor with the detergent-insoluble, cytoskeletal residue. Incubation of ligand-bound cells at 37 C promoted the degradation of surface Ig and the appearance of new, lower mw species, including a major soluble protein (50,000 daltons) that was antigenically related to surface Ig. These events corresponded to receptor endocytosis by several criteria (time course, temperature sensitivity, and energy dependence). Taken together, these results were consistent with the ligand-induced transmembrane attachment of receptors to the underlying cytoskeletal matrix followed by receptor internalization and catabolism. (Author abstract) (41 Refs)

45. A MONOCLONAL ANTIBODY THAT DETECTS EXPRESSION OF TRANSFERRIN RECEPTOR IN HUMAN ERYTHROID PRECURSOR CELLS.

Lebman D, Trucco M, Bottero L, Lange B, Pessano S, Rovera G (c/o Rovera), Wistar Inst. Anatomy and Biology, 36th St. at Spruce, Philadelphia, PA, 19104
Blood; 59(3):671-678 1982

A monoclonal antibody, L5.1, obtained by immunizing a Balb/c mouse with HL60 human promyelocytic leukemia cells, was found to react with both HL60 cells and with the K562(S) cell line. This monoclonal antibody binds and immunoprecipitates a glycoprotein (Mr 87,000) present on the cell surface membrane of K562(S) as a disulfide bonded dimer. In competition experiments L5.1 competes with both transferrin and OKT9 (a known antitransferrin receptor antibody) for binding to target K562(S) erythroleukemia cells. Binding of both L5.1 and transferrin to the surface of K562(S) cells is inhibited by treatment with 12-O-tetradecanoyl-phorbol-13-acetate, and the extent and time course of inhibition is similar in both cases. Cell sorting analysis of normal human marrow cells incubated with L5.1 indicates that L5.1 reacts strongly with all the morphologically recognizable erythroid lineage precursors, from the pronormoblast to the orthochromatic normoblast, and with reticulocytes. Erythrocytes, myeloid element s, monocytes, megakaryocytes and platelets, peripheral blood B and T lymphocytes do not bind significantly with this antibody and only a small fraction of promyelocytes was reactive. Antibody L5.1 did not react with leukemic cells of patients with acute lymphoblastic, myeloblastic and promyelocytic leukemias, but it did react with some established B (1 of 5) and T (2 of 3) cell lines, and a myeloid (1 of 3) cell line, and with PHA-stimulated peripheral blood lymphocytes. The nonhemopoietic cell lines tested did not bind with L5.1 with the exception of a colorectal adenocarcinoma and a melanoma cell line, which were both strongly positive. The relationship of antibody L5.1 to other monoclonal antibodies that bind the transferrin receptor is discussed. (Author abstract) (35 Refs)

46. PROTEINS ANTIGENICALLY RELATED TO THE HUMAN ERYTHROCYTE GLUCOSE TRANSPORTER IN NORMAL AND ROUS SARCOMA VIRUS-TRANSFORMED CHICKEN EMBRYO FIBROBLASTS.

Salter DW, Baldwin SA, Lienhard GE, Weber MJ
Dept. Microbiology and Public Health, Michigan State Univ., East Lansing, MI, 48823
Proc Natl Acad Sci USA; 79(5):1540-1544 1982

Antibody raised against the purified human erythrocyte glucose transporter specifically precipitated four proteins from normal and Rous sarcoma virus-transformed chicken embryo

cells: a major protein of Mr 41,000 and minor proteins of Mr 68,000, 73,000, and 82,000. The Mr 41,000 and 82,000 proteins were found only in a membrane fraction, not in the soluble fraction, and displayed a heterogeneous mobility on Na-DodSO4/polyacrylamide gel electrophoresis, suggesting glycosylation. The Mr 41,000 and 82,000 proteins were increased in amount after malignant transformation in direct proportion to the increase in hexose transport rate, and the increase was dependent on the expression of the src gene product, as revealed with a temperature-conditional src mutant. We suggest that the Mr 41,000 and 82,000 proteins are the glucose transporter of chicken embryo fibroblasts, or a component of the glucose transporter. These experiments provide direct evidence that malignant transformation increases the rate of glucose transport by increasing the number of transporters in the membrane. (Author abstract) (27 Refs)

47. DEFECTIVE INTERNALIZATION OF LOW DENSITY LIPOPROTEIN IN EPIDERMOID CERVICAL CANCER CELLS.

Gal D, Simpson ER, Porter JC, Snyder JM
Cecil H. and Ida Green Center for Reproductive Biology Sciences, Dallas, TX, 75235
J Cell Biol; 92(3):597-603 1982

Cells of an epidermoid cancer cell line of human uterine cervix, which possessed a high-affinity, specific receptor for low density lipoprotein (LDL), internalized and degraded [125I]iodo-LDL at a very low rate. In these cells, LDL did not stimulate cholesteryl ester synthesis, nor did it suppress 3-hydroxy-3-methylglutaryl coenzyme A reductase to the same extent as an the con trol cells. The binding of [125I]iodo-LDL by these cells was not decreased by preincubation of the cells in medium containing LDL. Using ferritin-labled LDL (F-LDL) and electron microscopy, it was determined that at 4 C the cells bound F-LDL in the same way as other cancer cell lines that did not have a defect in internalization. When these cells were warmed to 37 C the F-LDL remained on the surface, whereas in cells from control cancer cell lines the F-LDL was internalized and was no longer observed on the cell surface. On the basis of the results of these studies it is concluded that cells of this epidermoid cancer cell line have a defective ability to internalize LDL. (Author abstract) (26 Refs)

48. APAMIN AS A SELECTIVE BLOCKER OF THE CALCIUM-DEPENDENT POTASSIUM CHANNEL IN NEUROBLASTOMA CELLS: VOLTAGE-CLAMP AND BIOCHEMICAL CHARACTERIZATION OF THE TOXIN RECEPTOR.

Hugues M, Romey G, Duval D, Vincent JP, Lazdunski M
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Proc Natl Acad Sci USA; 79(4,part1):1308-1312 1982

This paper describes the interaction of apamin, a bee venom neurotoxin, with the mouse neuroblastoma cell membrane. Voltage-clamp analyses have shown that apamin at low concentrations specifically blocks the Ca++-dependent K+ channel in differentiated neuroblastoma cells. Binding experiments with highly radiolabeled toxin indicate that the dissociation constant of the apamin-receptor complex in differentiated neuroblastoma cells is 15-22 pM and the maximal binding capacity is 12 fmol/mg of protein. The receptor is destroyed by proteases, suggesting that it is a protein. The binding capacity of neuroblastoma cells for radiolabeled apamin dramatically increases during the transition from the nondifferentiated to the differentiated state. The number of Ca++-dependent K+ channels appears to be at most 1/5 the number of fast Na+ channels in differentiated neuroblastoma. The binding of radiolabeled apamin to its receptor is antagonized by monovalent and divalent cations. Na+ inhibition of the binding of 125I-labeled apamin is of the competitive type (Kd(Na+) = 44 mM). Guanidinium and guanidinated compounds such as amiloride or neurotensin prevent binding of 125I-labeled apamin, the best antagonist being neurotensin. (Author abstract) (22 Refs)

TRANSPORT STUDIES

49. PHOSPHATE CONCENTRATION AND TRANSPORT IN EHRLICH ASCITES TUMOR CELLS: EFFECT OF SODIUM.

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Dept. Physiology, Univ. Texas Health Science Center at San Antonio, San Antonio, TX, 78284
J Cell Physiol; 110(2):149-154 1982

Factors influencing inorganic P (Pi) transport in Ehrlich-Lettre ascites tumor cells (hyperdiploid strain), particularly the Pi and Na+ composition of the medium, were studied in an attemp t to characterize mechanisms responsible for regulation of the intracellular Pi content. The possibility that hydrolysis of organic phosphate esters contributes to the chemically determined estimate of Pi was examined. Pi transport, as well as the intracellular Pi concentration, showed a marked dependence on the Na+ concentration of the extracellular environment, implying that transport plays a principle role in maintaining the cellular Pi concentration. (19 Refs)

50. CA++-DEPENDENT K+ TRANSPORT IN THE EHRLICH ASCITES TUMOR CELLS.

Valdeolmillos M, Garcia-Sancho J, Herreros B
Departamento de Fisiologia y Bioquimica, Facultad de medicina, Valladolid, Spain
Biochim Biophys Acta; 685(3):273-278 1982

The possible presence and properties of the Ca++-dependent K+ channel have been investigated in the Ehrlich ascites tumor cell. The treatment with ionophore A23187 + Ca++, propranolol or the electron donor system ascorbate-phenazine methosulphate, all of which activate that transport system in the human erythrocyte, produces in the Ehrlich cell a net loss of K+ (balanced by the uptake of Na+) and a stimulation of both the influx and efflux of 86Rb. These effects were antagonized by quinine, a known inhibitor of the Ca++-dependent K+ channel in other cell systems, and by the addition of EGTA to the incubation medium. Ouabain did not have an inhibitory effect. The results suggest that the Ehrlich cell possesses a Ca++-dependent K+ channel whose characteristics are similar to those described in other cell systems. (Author abstract) (29 Refs)

51. ION TRANSPORT IN YEAST.

Borst-Pauwels GW
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Biochim Biophys Acta; 650(2/3):88-127 1981

Kinetics and energization of inorganic ion transport in yeast are discussed. Mathematical and methodological approaches to surface potential, cell pH, and membrane potential are related

to inorganic ion transport in yeast. The review concerns mainly the uptake of monovalent cations, divalent cations, phosphate, and sulfate in the yeast system. (348 Refs)

52. CORRELATION BETWEEN THE RATES OF AEROBIC GLYCOLYSIS AND GLUCOSE TRANSPORT, UNRELATED TO NEOPLASTIC TRANSFORMATION, IN A SERIES OF BALB 3T3-DERIVED CELL LINES.

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Cancer Res; 42(5):1809-1816 1982

The relationship between transformation, lactate production, and glucose transport was examined in a series of 10 cell lines consisting of subclones of BALB 3T3 A31 cells and viral and chemical transformants of either the subclones or the original A31 line. Comparisons were made over a relatively narrow range of cell densities to minimize changes in the biochemical parameter during growth. A nitr oquinoline oxide (NQT-3T3-714) and a temperature-sensitive Kirsten sarcoma virus (tsKi-3T3-714) transformant of subclone 714 exhibited transformed phenotypes with respect to morphology and growth properties, but their rates of lactate production and 2-[3H]deoxy-D-glucose (deoxyglucose) uptake were similar to those of the parent cells. Inhibitors of oxidative phosphorylation increased glycolysis fold in these transformants, showing that there was no defect in the enzymes of this pathway. At a temperature non-permissive for transformation of tsKi-3T3-714, lactate production by this line did not decrease relative to the rate of the parent cells. Another transformant, Ki-3T3-234, had a glycolytic rate which was 4 to 5x greater than that of the low lactate producers while other transformants exhibited intermediate rates, and the rate of a third nontransformed 3T3 A31 subclone, K-1-1, was comparable to the rate of Ki-3T3-234. The rates of [3H]deoxyglucose uptake by this series of cells were closely proportional to their glycolytic rates rather than to their state of transformation. Increasing glycolysis by oligomycin or dinitrophenol treatment, however, did not cause a concomitant increase in sugar uptake. Neither glycolysis nor deoxyglucose uptake in the high-lactate producer (Ki-3T3-234) was inhibited by ouabain, suggesting that Na+-K+ adenosinetriphosphatase is not a regulator of these functions in 3T3 cells. In 3T3-derived cells, it appears that the rates of glycolysis and glucose uptake may be regulated in tandem under some conditions and that neither process is an obligatory consequence of neoplastic transformation. (Author abstract) (31 Refs)

53. APPROACHES TO THE MEASUREMENT OF INTRACELLULAR ADENINE AND THE DISCRIMINATION BETWEEN ADENINE TRANSPORT AND METABOLISM IN L1210 LEUKEMIA CELLS.

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Biochim Biophys Acta; 714(3):435-441 1982

Incubation of L1210 leukemia cells with 10 uM [3H]adenine in the absence of energy substrate results in a very rapid accumulation of 3H within the cells. By 20 s intracellular adenine is near steady-state; beyond this the rate of accumulation of intracellular 3H reflects nucleotide synthesis, predominantly the rate of ATP accumulation within the cell as determined by liquid chromatography. Adenine incorporation into the nucleotides proceeds via adenine-phosphoribosyl transferase, which is rate-limiting to AMP formation and subsequently the formation of ADP and ATP. Acceleration of this pathway by the addition of glucose and phosphate decreases the intracellular adenine level far below equilibrium as metabolism is increased relative to transport. Assessment of methodology to evaluate intracellular adenine and it s metabolites indicates that (i) a 4 C wash removes the major portion of intracellular adenine and (ii) at 4 C, transport of adenine remains rapid and while nucleotide synthesis is decreased, ATP still accumulates within the cell. Hence, measurement of cellular uptake of radioactive label at 4 C after cells are washed free of adenine cannot be used as a measurement of adenine surface binding since this radioactive label represents, at least in part, phosphorylated derivatives of adenine within the cell. Unlabeled adenine and structurally related compounds were found to inhibit [3H]adenine net uptake under conditions where metabolism of adenine was reduced, suggesting that base transport is mediated by a facilitated diffusion mechanism. This is consistent with other studies from this laboratory that demonstrate exchange diffusion between adenine and other bases. (Author abstract) (21 Refs)

54. INITIAL RATE KINETICS OF THE TRANSPORT OF ADENOSINE AND 4-AMINO-7-(BETA-D-RIBOFURANOSYL)PYRROLO[2,3 D]PYRIMIDINE TUBERCIDIN IN CULTURED CELLS.

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Cancer Res; 42(4):1289-1295 1982

A procedure is described for determining early time courses of nucleoside uptake by cultured cells in suspension. Replicate samples of cell suspensions were exposed to medium containing 3H-nucleosides for brief intervals (sec) ended by addition of nitrobenzylthioinosine, a potent inhibitor of nucleoside transport that terminated nucleoside uptake virtually instantaneously. Time courses of nucleoside uptake were constructed from the cellular content of nucleoside acquired by the replicate samples during graded intervals of exposure to the labeled permeant. Such time courses were definitive of cellular uptake of nucleosides during the first few sec of exposure to permeant and yielded initial rates of uptake of adenosine and 4-amino-7-(beta-D-ribofuranosyl)pyrrolo[2,3 d]pyrimidine (tubercidin). Defining initial rates of nucleoside uptake as rates of inward transport, relationships between transport rates and extracellular concentrations of these permeants were evaluated in HeLa cells and in two cultured lines of mouse lymphoma L5178Y cells that differ in their abilities to phosphorylate adenosine and tubercidin. Transport rates for these permeants were similar in the two L5178Y cell types and were saturable in the three cell lines with Km values between 14 and 38 uM. Adenosine and tubercidin were mutually competitive permeants in L5178Y cells, indicating that they are substrates for the same transport mechanism. (Author abstract) (25 Refs)

55. CHARACTERISTICS OF DOXORUBICIN TRANSPORT IN HUMAN RED BLOOD CELLS.

Dalmark M
Finseninstitutet, medicinsk afdeling, Strandboulevarden 49, Copenhagen DK-2100, Denmark
Scand J Clin Lab Invest; 41(7):633-639 1981

The transport characteristics of doxorubicin (DR:adriamycin) efflux from human erythrocytes was measured by spectrophotofluorometric determination of the net efflux into DR-free medium at 37 C. Low concentrations of

phloretin, 1-alcohols, local anesthetics, HgCl2, LaCl3, p-chloromercuribenzoate, and pyridoxal-5-phosphate were added to the medium to examine their effects on DR efflux. DR efflux decreased with pH and increased with increasing chain length of 1-alcohols, with cationic, anionic, and electroneutral anesthetics, but was little affected by various inhibitors of facilitated transport systems, indicating that proteins are not involved in DR transport. The data are consistent with the perception of DR transport as a Fickean diffusion transport process of the electroneutral (unprotonated) DR molecule through the lipid domain of the cell membrane. (30 Refs)

56. UPTAKE, EFFLUX, AND HYDROLYSIS OF ACLACINOMYCIN A IN FRIEND LEUKEMIA CELLS.

Fourcade A, Farhi JJ, Bennoun M, Tapiero H
(c/o Tapiero) Dept. Pharmacologie Cellulaire, Institut de Cancerlogie et d'Immunogenetique (INSERM U-50), Unite Simone et Cino del Duca de Pharmacologie Humaine des Cancers, 94804 Villejuif, France
Cancer Res; 42(5):1950-1954 1982

The kinetics of uptake by cells and nuclear incorporation of aclacinomycin A was studied in Friend leukemia cells. It was shown that uptake is a very rapid process. The intracellular concentration is maximum in 10 min and mainly (about 75%) localized in the nucleus. Most of the incorporated drug will disappear from the cell by a two-step mechanism: (a) efflux from the nucleus to the cytoplasm; and (b) deglycosidation at C-7 to the alkavinone form in the cytoplasmic fraction. The cellular uptake was temperature dependent but was not prevented by sodium azide treatment. We assumed, therefore, that it is related to the composition and to the dynamic structure of the cell surface membrane. Nuclear outward transport and deglycosidation were inhibited by sodium azide and low temperatures; this suggests that they are regulated by an active transport process and by an enzymatic activity, respectively. (Author abstract) (21 Refs)

MEMBRANE BIOGENESIS AND METABOLISM

57. BIOSYNTHESIS AND PROCESSING OF A HUMAN T LYMPHOCYTE ANTIGEN.

Bergman Y, Levy R
Howard Hughes Medical Inst., Stanford Univ., Stanford, CA, 94305
J Immunol; 128(3):1334-1340 1982

The biosynthesis and processing of Leu-1, a human T lymphocyte antigen, has been studied with the use of a monoclonal antibody. This molecule exists on the cell surface as a 67,000 mw glycoprotein. Through a series of pulse-labeling studies, in conjunction with the use of the antibiotic tunicamycin and the enzyme Endo-H, the details of glycosylation, processing, and deposition at the cell membrane we re examined. The protein backbone of the molecule is 58,000 mw. High-mannose sugars are added to asparagine residues during synthesis. Within 20 min, these high mannose sugars are converted to complex type carbohydrates, including fucose. The fully processed glycoprotein appears at the cell surface within 30 min after synthesis. This sequence of events is similar to that for other cell surface glycoproteins, including HLA and vesicular stomatitis virus glycoprotein. (Author abstract) (35 Refs)

58. FORMATION OF THE ENVELOPE OF ROUS SARCOMA VIRUS AND VESICULAR STOMATITIS VIRUS FROM LOCALIZED LIPID REGIONS IN THE PLASMA MEMBRANE.

Omann GM, Cimino J, Pessin JE, Glaser M
Dept. Biochemistry, Univ. Illinois, Urbana, IL, 61801
Biophys J; 37(1):135-136 1982

The effects of altering the host cell plasma membrane lipid composition and fluidity on the viral envelope lipid composition and fluidity were determined in chick embryo fibroblasts treated with a Rous sarcoma virus mutant (RSV-T5). Results showed differences in the lipid composition of the plasma membrane from RSV-T5 infected cells grown at 36 C or 41 C. However results of 1,6-diphenyl-1,3,5-hexatriene (DPH) anisotropies showed identical virions produced by the cells. Steady state and limiting anisotropies of DPH in the supplemented plasma membranes and vesicular stomatitis virus envelopes were similar. The virus apparently selectively incorporates host cellular phospholipids into the viral envelopes. (6 Refs)

CELL ADHESION

59. THE COOPERATIVE ROLE OF THE TRANSFORMATION-SENSITIVE GLYCOPROTEINS, GP140 AND FIBRONECTIN, IN CELL ATTACHMENT AND SPREADING.

Carter WG
Biochemical Oncology, Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
J Biol Chem; 257(6):3249-3257 1982

The detergent-insoluble matrix of cultured human fibroblasts contains cytoskeletal and nuclear components, as well as two major, noncollagenous glycoproteins, fibronectin and GP140. These glycoproteins are stabilized by extensive intermolecular disulfide bonding. GP140 was partially purified, and a higher yield of GP140 could be obtained under reducing conditions by extraction with urea-dithiothreitol followed by molecular sieve chromatography. The purified GP140 contained mannose, galactose, and N-acetylgucosamine residues. Amino acid analysis of GP140 detected periodate-sensitive residues, as well as hydroxylproline. These hydroxylated amino acids are major components of collagens and collagen-like proteins. The GP140 isolated under nonreducing conditions was found to induce stable cell attachment and cell spreading when coated on plastic surfaces. The cell attachment could not be inhibited with affinity purified anti-fibronectin antibodies. However, trypsinization of cells under conditions that removed surface fibronectin reduced the ability of the cells to bind to the GP140-coated surface. Metabolic labeling of cells with radioactive glucosamine during 1-hr cell attachment experiments incorporated label into both fibronectin and GP140, indicating that the cells rapidly glycosylate both fibronectin and GP140 and incorporate them into the stable detergent-insoluble matrix. Long term labeling and chase experiments indicated that fibronectin and GP140 in the matrix are subject to very slow turnover. Neither fibronectin nor GP140 were detectable in the detergent-insoluble matrix of SV40-transformed human fibroblasts by either metabolic or cell surface labeling. These results are consistent with the conclusion that fibronectin and GP140 may function in a cooperative manner in cell adhesion and spreading. (70 Refs)

60. FIBRONECTIN DISTRIBUTION PATTERN AND THE THREE-DIMENSIONAL GROWTH BEHAVIOR OF MAMMALIAN CELLS UNDER ANCHORAGE-INDEPENDENT CONDITIONS.

Rajaraman R, Lonergan K
Dept. Microbiology, Dalhousie Univ., Halifax, Nova Scotia B3H 4H7, Canada
Exp Cell Biol; 50(1):2-12 1982

Several types of normal diploid cells, established 'normal' cells, and transformed cells of human and rodent origin have been studied with reference to cell surface fibronectin distribution and their anchorage-independent growth behavior. All cell types that showed intercellular and fibrillar surface fibronectin were anchorage-dependent for growth. Lack of surface fibrillar fibronectin in spontaneously or virus-transformed cells and cells of neoplastic origin was accompanied by anchorage-independent growth potential. The degree of three-dimensional organization of cells under anchorage-independent growth conditions was dependent on the amount of intercellular fibronectin. The significance of these observations to in vivo tumor growth is discussed. (Author abstract) (21 Refs)

61. STRIPPING OF EXTERNAL CELL-ASSOCIATED FIBRONECTIN FROM MITOTIC FIBROBLASTS.

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Cell Struct Funct; 6(4):313-327 1981

The timing of release of fibronectin (FN) according to cell cycle phase was studied in the C3H2K mouse fibroblastic cell line using trypsinization to stimulate arrested cells and hydroxyurea (HU) to prevent progression of the cell cycle in trypsinized cells. HU-treated and untreated cells showed no significant difference in FN release, suggesting that FN release into the medium is not cell-cycle dependent. Immunofluorescence showed that mitotic cells tend to become round and leave FN networks on the surface of the substratum surrounding the point where they were attached. (26 Refs)

62. RETINOIC ACID INDUCES ANCHORAGE- AND DENSITY-DEPENDENT GROWTH WITHOUT RESTORING NORMAL CYTOSKELETON, EGF BINDING, FIBRONECTIN CONTENT AND ODC ACTIVITY IN A RETROVIRUS-TRANSFORMED MOUSE CELL LINE.

Mukherjee BB, Mobry PM, Pena SD
Dept. Biology, Center Human Genetics, Montreal, Quebec H3A 1B1, Canada
Exp Cell Res; 138(1):95-107 1982

We studied the effects of retinoic acid (RA), a known tumor inhibitor, on a group of transformation-related properties expressed by a Kirsten sarcoma virus (Ki-MSV)-transformed BALB/c 3T3 (clone A31) cell line (KA31). The aim was to determine whether any one or a group of such transformation-specific changes, exhibited by this cell line, is an absolute prerequisite for its acquisition of the neoplastic state. Our study shows that RA treatment restores two important parameters of normalcy in KA31 cells: density-dependent growth inhibition, and anchorage-dependent growth. Retinoic acid also induces cell-to-substratum adhesiveness in a significant proportion of the treated KA31 cell population. However, four other markers of transformation - loss of microfilaments, lack of surface fibronectin, reduced epidermal growth factor (EGF) binding, and increased ornithine decarboxylase activity - remain unaltered by RA treatment. These transformation-specific changes, therefore, are dissociable from loss of anchorage- and density-dependent growth. It is suggested that RA may intervene in transforming protein kinase-induced cell surface changes that are involved in cellular malignancy and the loss of growth control in KA31 cells. (Author abstract) (57 Refs)

63. DEPOSITION OF BASEMENT MEMBRANE PROTEINS IN ATTACHMENT AND NEURITE FORMATION OF CULTURED MURINE C-1300 NEUROBLASTOMA CELLS.

Alitalo K, Kurkinen M, Virtanen I, Mellstrom K, Vaheri A
Dept. Virology, Univ. Helsinki, Haartmaninkatu 3, SF-00290 Helsinki 29, Finland
J Cell Biochem; 18(1):25-35 1982

The deposition of basement membrane (BM) glycoproteins during the processes of substrate attachment and cell differentiation was studied in cultures of C-1300 mouse neuroblastoma cells (clone NB41A3). Cell differentiation was induced by sparse plating and the addition of dibutyryl cyclic AMP (dbcAMP: 1 milliM) to the medium. Indirect immunofluorescence microscopy disclosed a typical perinuclear cytoplasmic staining for BM antigens in both differentiated and undifferentiated cells. No surface fluorescence was detected in freshly seeded cells in suspension, but punctate deposits of laminin were detected on the ventral surfaces of the cells within 2 hr after plating. In sparsely seeded cultures of undifferentiated C-1300 cells, extracellular laminin deposits were detected only under the primary attaching cells, not the daughter cells in the clonal colonies. In dbcAMP-exposed cultures, laminin deposition was detected in association with the growing cytoplasmic extensions of the newly formed neurites. The secreted proteins of cultured differentiated and undifferentiated neuroblastoma cells showed markedly different patterns of fucosylation of the high-mol wt proteins. No deposits of fibronectin or Type IV collagen were found under the attaching and a differentiating cells. It is concluded that cultured C-1300 cells can synthesize, secrete, and deposit laminin. The distribution of laminin during cell attachment and neurite extension suggests that laminin may be involved in cell-to-substrate interactions in cultured C-1300 neuroblastoma cells. (53 Refs)

64. TERATOCARCINOMA CELL ADHESION: IDENTIFICATION OF A CELL-SURFACE PROTEIN INVOLVED IN CALCIUM-DEPENDENT CELL AGGREGATION.

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Dept. Biophysics, Faculty Science, Kyoto Univ., Kitashirakawa, Sakyo-ku, Kyoto 606, Japan
Cell; 28(2):217-224 1982

Teratocarcinoma cells have a Ca++-dependent cell-cell adhesion site (t-CDS) that is unique in being inactivated with trypsin in the absence of Ca++ but not in the presence of Ca++. Fab fragments of antibodies raised against teratocarcinoma F9 cells dissociated by treatment with trypsin and calcium (anti-TC-F9) inhibit the aggregation of teratocarcinoma cells mediated by t-CDS. This inhibitory effect of Fab is removed when anti-TC-F9 is absorbed with F9 cells treated with trypsin and calcium (TC-F9), but not when it is absorbed with F9 cells treated with trypsin and EGTA (TE-F9). Comparisons of cell-surface antigens reactive to anti-TC-F9 in TC-F9 cells with those in TE-F9 cells reveal that only one component, with an approx mol wt of 140,000 (p140), is detected specifically on the surface of TC-F9 cells. When TC-F9 cells are retrypsinized in the absence of Ca++, a substance with an

approx mol wt of 34,000 (p34) is released that can neutralize the aggregation-inhibitory effect of the Fab. This p34 interferes with the immunoprecipitation of p140 with anti-TC-F9, suggesting that p34 is a tryptic fragment of p140. Anti-TC-F9 Fab causes the dissociation of the monolayers of teratocarcinoma cells. This effect is removed by absorption of the Fab with p34 as well as with TC-F9 cells, but not with TE-F9 cells. These results suggest that p140 is essential for the function of t-CDS, and that this is an actual cell-adhesion molecule active in the establishment of monolayers of teratocarcinoma cells. (Author abstract) (37 Refs)

65. INTEGRAL MEMBRANE GLYCOPROTEINS RELATED TO CELL-SUBSTRATUM ADHESION IN MAMMALIAN CELLS.

Damsky CH, Knudsen KA, Buck CA
Wistar Inst. Anatomy and Biology, Philadelphia, PA, 19104
J Cell Biochem; 18(1):1-13 1982

Goat antisera were raised against surface membrane-derived material from baby hamster kidney cells (BHK21/C13), a transformed line derived from cell line (C13/B4), and mouse mammary tumor epithelial cells (BALB/c MT2). The antisera to the hamster cell lines (anti-M) and the MT2 cells (anti-GP) disrupted cell-substrate adhesions in cultures of their respective cell types. An antibody blocking assay was used to isolate adhesion-related glycoproteins (GP) from non-ionic detergent (Nonidet NP40) extracts of each cell type. In each case, the product was a restricted population of GPs (mol wt about 120,000-160,000). Each GP fraction blocked the disruption of adhesion induced by the goat antiserum on either cell type (hamster kidney or mouse mammary tumor). The antisera were capable of disrupting cell-substratum adhesion of a large number of cell types and species sources. Antibody-blocking activity was detected in partially purified extracts of a variety of cultured cell types. In addition to similar substrate-associated GPs (eg, fibronectin) and cytoskeleton-associated proteins (eg, alpha-actinin and vinculin), cells from different tissues and different species apparently contained a relatively conserved class of integral membrane GPs involved in cell-substrate adhesion. (29 Refs)

66. CELLULAR ADHESION TO GROWTH SURFACES: A POSSIBLE ROLE FOR LOW MOLECULAR WEIGHT SURFACE COMPONENTS.

Wildridge M, Sherbet GV
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Exp Cell Biol; 50(1):27-33 1982

Cell lines initiated from a fibrocystic hyperplasia and a carcinoma of the breast and an established human fetal lung fibroblast line, MRC5, were grown in culture. The surface proteins of the cells were iodinated in cell monolayer, and in suspension following harvesting from culture flasks using EDTA. Data are presented which show that cells labeled in suspension show reduced incorporation of 125I into surface proteins of high mol wt (greater than 100,000 daltons) and considerably increased incorporation into proteins with mol wt less than 100,000 daltons. These changes are discussed in relation to the possible release of surface material by the action of EDTA. It is also suggested, on the basis of the current findings, that the detachment of cells may expose low mol wt components possibly involved in the attachment of the cells to the substratum. (Author abstract) (18 Refs)

OTHER RELATED STUDIES

67. ENDO- AND ECTOCERVICAL HUMAN UTERINE EPITHELIAL CELLS DISTINGUISHED BY FIBRONECTIN PRODUCTION AND KERATINIZATION IN CULTURE.

Alitalo K, Halila H, Vesterinen E, Vaheri A
Dept. Virology, Univ. Helsinki, Haartmaninkatu 3, 00290 Helsinki 29, Finland
Cancer Res; 42(3):1142-1146 1982

Human ecto- and endocervical uterine epithelia grow in culture as stratifying and nonstratifying cell layers, patterns reflecting the differentiation of the corresponding cells in vivo. We found that both cultures synthesized and secreted fibronectin but very little other known glycoproteins of basal laminae, laminin, and Type IV collagen. Fibronectin production was observed only in the lining marginal cells of the expanding periphery of ectocervical islands, but differentiating and keratinizing cells had lost fibronectin. Most cells in endocervical epithelial cultures grew as a monolayer and produced fibronectin. After stratification of the ectocervical cells, the cytokeratin polypeptide pattern switched to the large-mol-wt type. In endocervical cultures, the low-mol-wt keratin polypeptides persisted. Fibronectin production thus appears to be linked to the state of differentiation of the epithelial cells, and fibronectin may function in the attachment of the basal cells to underlying basal lamina matrix. (Author abstract) (37 Refs)

68. CYTOSKELETAL INFLUENCE ON MEROCYANINE 540 RECEPTORS IN THE PLASMA MEMBRANE OF ERYTHROLEUKEMIC CELLS.

Hunt RC, Hood JA
Dept. Biochemistry, Univ. Mississippi Medical Center, 2500 North State St., Jackson, MS, 39216
Biochim Biophys Acta; 720(1):106-110 1982

When human erythroleukemic cells are induced to differentiate in vitro, the lipids in the plasma membrane that bind the fluorescent dye merocyanine 540 are redistributed into a cap at one pole of the cell. This capping phenomenon can also be observed in uninduced cells that have been incubated with cytochalasin B, an agent which disrupts actin-containing microfilaments or with local anesthetics which act on both microfilaments and microtubules. Colchicine which acts on microtubules, however, has no effect. This suggests that the uniform distribution seen in uninduced cells is maintained by the cytoskeletal microfilaments and that loss of these structures leads to spontaneous redistribution of merocyanine 540-binding sites. (Author abstract) (18 Refs)

69. PHOSPHORYLATION OF VINCULIN BY PP60SRC. WHAT MIGHT IT MEAN?

Hynes R
Center for Cancer Res., Massachusetts Inst. Technology, Cambridge, MA, 02139
Cell; 28(3):437-438 1982

Alterations in tyrosine kinase-specific vinculin following infection with Rous sarcoma virus pp60src are described. Vinculin is concentrated at sites of actin-membrane interaction and cell-substratum contact. Pp60src, the transforming protein of Rous sarcoma virus, is concentrated in the same regions. Changes in vinculin, alpha-actinin, and microfilament bundles following transformation are described. (no Refs)

70. CORRELATION BETWEEN AMOUNTS OF CELLULAR MEMBRANE COMPONENTS AND SENSITIVITY TO HYPERTHERMIA IN A VARIETY OF MAMMALIAN CELL LINES IN CULTURE.

Cress AE, Culver PS, Moon TE, Gerner EW

Dept. Radiology, Div. Radiation Oncology, Arizona Health Sciences Center, Tucson, AZ, 85724

Cancer Res; 42(5):1716-1721 1982

The wt ratio of either cholesterol or phospholipid to protein contents in seven different cell lines, growing exponentially at 37 C, correlates positively with increasing resistance of the cells to subsequent hyperthermic cell killing. The relative heat resistance of each cell line is derived from survival curves obtained when the different cell lines are exposed to 43 C. Cholesterol and phospholipid amounts in the particulate fraction correlate with survival sensitivity to 43 C when the values are expressed per mg protein but not when expressed per cell number. Also, cholesterol:phospholipid molar ratios and the amount of protein in the particulate fraction do not display linear correlations with sensitivity of the respective cell lines to 43 C-induced cell killing. The relative degree of fatty acid saturation at 37 C also is independent of whether cells show a higher degree of heat resistance. These data suggest that lipid (both cholesterol and phospholipid):protein weight ratios correlate with increasing resistance of cells to an elevation in temperature. The major implication of these data is that major membrane components can influence and perhaps predict cellular survival to hyperthermia. (Author abstract) (28 Refs)

71. ACTION OF IONIZING RADIATION ON LIPID-PROTEIN INTERACTION.

Verma SP, Wallach DF

Therapeutic Radiology Dept., Radiobiology Div., Tufts-New England Medical Center, 171 Harrison Ave., Boston, MA, 02111

Biochem Biophys Res Commun; 105(1):105-109 1982

The effect of 137Cs gamma-irradiation (100 rad) on lipid-protein interactions was investigated by laser Raman spectroscopy. Liposomes composed of myelin proteolipid apoprotein (PLA) + dipalmitoylphosphatidylcholine + linoleic acid were used as a model membrane system. Results suggest that irradiation generates linoleic acid hydroperoxides which combine with PLA, resulting in different phase characteristics for the system. However, PLA itself may be damaged by the radiation, with the altered PLA molecule reacting differently with the lipid phase. (11 Refs)

72. ANTIBODY MEDIATED DELIVERY OF TOXIC MOLECULES TO ANTIGEN BEARING TARGET CELLS.

Raso V

Sidney Farber Cancer Inst., 44 Binney St., Boston, MA, 02115

Immunol Rev; 62:93-117 1982

The ability of hybrid antibodies with dual specificity to deliver toxic agents to immunoglobulin (Ig)-bearing target cells is reviewed. Progress on both the Ig and CALLA (conjugates which are selectively cytotoxic for human cells bearing the acute lymphoblastic leukemia antigen) are discussed. Topics discussed include hybrid antibodies with simultaneous cell surface and toxic agent binding activity (anti-neocarzinostatin, anti-human IgG hybrids and anti-ricin A chain/anti-human IgG hybrids) and selectively cytotoxic antibody-ricin A chain conjugates (human Ig directed antigen binding fragment [F(ab')]-A chain conjugate, monoclonal human kappa-chain specific F(ab')2-A chain conjugate, and monoclonal CALLA-specific F(ab')-A chain conjugates). Hybrid-mediated enhancement of toxicity has been achieved using neocarzinostatin , whole ricin, and free ricin A chain. (43 Refs)

73. A NEW INTERCELLULAR COMMUNICATION ASSAY: ITS USE IN STUDIES ON THE MECHANISM OF TUMOUR PROMOTION.

Fitzgerald DJ, Murray AW

Sch. Biological Sciences, Flinders Univ., Bedford Park, 5042, South Australia

Cell Biol Int Rep; 6(3):235-242 1982

A rapid, quantifiable technique capable of measuring the transfer of nucleotides between cells over periods as short as 30 min is reported. The method was used to extend previous work showing inhibition of intercellular communication by tumor-promoting phorbol esters. When cocultures of mouse epidermal cells (HEL-37) and mutant mouse fibroblast cells (PG-19) were incubated in 3 milliM EDTA, essentially all PG-19 cells and approx 20% of the HEL-37 cells were released, whereas cultures of HEL-37 cells alone released only about 2% of the cells. The extra HEL-37 cells released in coculture were probably those in contact with PG-19 cells, and were presumably involved as donor cells for the junctional transfer of labeled nucleotides. Results of a 3-hr autoradiographic analysis using 3H-hypoxanthine indicated that approx 33% of the released PG-19 cells were labeled by such transfer. Further results indicated that the av incorporation of 3H-thymidine and 14C-hypoxanthine per HEL-37 cell was the same in cells released from cocultures by EDTA treatment and those maintained in culture alone. Thus, alteration in the 3H:14C ratio in the released cells provides a direct measure of the transfer of labeled nucleotides from HEL-37 donor cells to PG-19 recipient cells, presumably via gap junctions. After a short lag period, continuous transfer of radioactivity to the PG-19 cells occurred for at least 3 hr. Transfer was inhibited by 12-O-tetradecanoylphorbol 13-acetate. (9 Refs)

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. ALTERATIONS IN HEPATIC 5'-NUCLEOTIDASE IN THE TUMOR-BEARING RAT.

Goodlad GA, Clark CM
Dept. Biochemistry and Microbiology, Univ. St. Andrews, Fife KY16 9AL, Scotland
Enzyme; 27(2):119-123 1982

The effect of the growth of the Walker 256 carcinoma on the level of 5'-nucleotidase and alkaline phosphatase in the whole liver and in an isolated hepatocyte membrane preparation of its host was investigated. Alkaline phosphatase activities of whole liver and plasma membrane were increased approx five-fold by tumor growth. A 50% decrease in whole liver 5'-

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nucleotidase activity was observed in tumor-bearing rats while the 5'-nucleotidase activity per milligram membrane protein was unaltered. Tumor growth would therefore appear to affect a pool of 5'-nucleotidase which is not associated with the plasma membrane. (Author abstract) (21 Refs)

2. DISTRIBUTION OF 5'-NUCLEOTIDASE DURING HEPATOCARCINOGENESIS INDUCED BY HEXACHLOROCYCLOHEXANE IN SWISS MICE.

Bhatt DK, Nigam SK, Lakkad BC, Babu KA, Karnik AB, Thakore KN, Kashyap SK, Chatterjee SK
Natl. Inst. Occupational Health (ICMR), Meghani Nagar, Ahmedabad 380 016, India
Indian J Exp Biol; 19(12):1159-1162 1981

Male inbred Swiss mice were fed 500 ppm of hexachlorocyclohexane (BHC) for 2, 4, or 6 mo and the livers were histochemically studied for the distribution of 5'-nucleotidase (5NT). After 2 mo, hepatocytes in the periportal area showed intense enzymatic activity. Activity in the nodular part increased compared with the nonnodular part. Tumor tissue always showed very strong 5NT activity compared with the nontumor tissue. Intercellular spaces showed strong 5NT activity. As time increased, the main changes in 5NT distribution were disappearance of 5NT from the cytoplasm (persisting even after tumor development), increase in 5NT activity in the nucleus (disappearing in well developed nodules and tumors), and gradual increase in 5NT activity on the plasma membrane and intercellular space. The change in activity during hepatocarcinogenesis may be associated with the functional and constitutional aspect of the plasma membrane. The absence of cytoplasmic activity of 5NT may be associated with complete disruption of ergestroplasm during carcinogenesis, excessive accumulation of glycogen in the cytoplasm, lysosomal damage, and repression of the gene responsible for synthesis of cytoplasmic 5NT. 5NT may be a specific indicator of hepatic diseases and tumors of the liver. (22 Refs)

3. HUMAN THY-1: EXPRESSION ON THE CELL SURFACE OF NEURONAL AND GLIAL CELLS.

Kemshead JT, Ritter MA, Cotmore SF, Greaves MF
Membrane Immunology Lab., Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
Brain Res; 236(2):451-461 1982

The expression of the cell surface glycoprotein Thy-1 in a variety of human neuronal and neuroglial cell lines and fresh neuronal tumor tissue was examined using immunofluorescent, radioimmune binding and quantitative absorption techniques. Amount and distribution of Thy-1 were examined. Human Thy-1 was identified with a rabbit anti-pure rat brain Thy-1 antiserum, and the extent to which its antigenic determinants are shared with rodent Thy-1 was analyzed. Thy-1 was present on the cell surfaces of 4 human neuronal lines, 2 glial cell lines, and most fresh neuroblastoma material (both primary tumor and bone marrow metastases), but with considerable heterogeneity of antigen expression. Binding of rabbit anti-rat brain Thy-1 antibodies was specific for the Thy-1 molecule: binding was completely blocked by prior absorption of the antibody with either human or rat pure Thy-1. Approx 25% f the total anti-rat Thy-1 antibodies in the reagent will bind to the human homolog of Thy-1. The Thy-1 antigen in humans seems to be associated with neuronal cells, as in rats and mice, although the antigen may be more common in the human nervous system. (27 Refs)

4. IMMUNOLOGIC RECOGNITION OF CELL SURFACE ANTIGENS IN NORMAL MOUSE NEURAL TISSUES AND IN NEUROEPITHELIAL CELLS OF THE OTT-6050 MOUSE TERATOMA. A RADIOMETRIC, GEL ELECTROPHORETIC, AND MORPHOLOGIC (IMMUNOFLUORESCENCE AND IMMUNOPEROXIDASE) STUDY.

Ramsay PB, Vandenberg SR, Eng LF, Herman MM, Rubinstein LJ
(c/o Rubinstein) Div. Neuropathy, Dept. Pathology, Univ. Virginia Sch. Medicine, Charlottesville, VA, 22908
Acta Neuropathol (Berl); 56(3):214-224 1982

Use of the immunoperoxidase method for recognition of neural cell surface antigens (SA) was studied. Membrane fractions were prepared from neural tissues, liver, spleen, kidney, heart, testes, and thymus of adult and neonatal F1(129/J x C3H/HeJ) mice and from three OTT-6050 mouse teratoma-derived tumors. Antiserum to neural SA was produced in New Zealand white rabbits by injection of membrane preparations. The antiserum cross-reacted with SA of neuroblasts and primitive and mature glial cells, as indicated by radioimmunoassays. Binding of the antiserum to neonatal brain membranes was almost completely eliminated by preabsorption on adult mouse brain membranes; binding was not reduced by preabsorption on liver, spleen, kidney, or heart membranes. Antiserum cross-reacted with certain non-neural tissue types including sperm and sperm-precursor cells, some epithelial cells in the medulla of adult mouse thymus, and various mesenchymal cells in neonatal mice. Binding of antiserum to brain membranes was slightly reduced by preabsorption with testis pellets or thymus cells. Cross-reactivity was eliminated by preabsorption with adult brain membranes. Preabsorption with testis pellets or thymus cells reduced staining of primitive neuroepithelial cells in teratoma-derived tumors; staining of neural cells in the mature neuropil was reduced but not eliminated. Polyacrylamide gel electrophoresis identified major protein bands at mol wt 26,000, 32,000, 40,000, 42,000, 45,000, and 54,000 daltons. Results of autoradiographic analysis indicated that the antiserum reacted only with minor protein components of mouse brain membranes. (38 Refs)

5. STRUCTURAL HOMOLOGY OF A MACROPHAGE DIFFERENTIATION ANTIGEN AND AN ANTIGEN INVOLVED IN T-CELL-MEDIATED KILLING.

Kurzinger K, Ho MK, Springer T
Lab. Membrane Immunochemistry, Sidney Farber Cancer Inst., Harvard Medical Sch., Boston, MA, 02115
Nature; 296(5858):668-670 1982

The cellular distribution and molecular structure of two murine cell-surface antigens, macrophage differentiation antigen 1 (Mac-1) and lymphocyte function-associated antigen 1 (LFA-1) were investigated. Mac-1 is expressed on macrophages, natural killer cells and 50% of bone marrow cells, but not on B or T lymphocytes. LFA-1 is expressed on B and T lymphocytes and 75% of bone marrow cells, but not on macrophages. Mac-1 and LFA-1, which share some common antigenic determinants recognized by monoclonal antibodies, comprise a novel family of leukocyte differentiation antigens. The two antigens have alpha-chains of mol wt 170,000 and 180,000, respectively; both have beta-chains of mol wt 95,000. Cross-linking studies indicate a non-covalent association of the subunits into an alpha-1-beta-1 quaternary structure. (11 Refs)

6. MURINE CELL SURFACE GLYCOPROTEINS. IDENTIFICATION, PURIFICATION, AND CHARACTERIZATION OF A MAJOR GLYCOSYLATED COMPONENT OF 110,000 DALTONS BY USE OF A MONOCLONAL ANTIBODY.

Hughes EN, August JT
(c/o August) Dept. Pharmacology and Experimental Therapeutics, Johns Hopkins Univ. Sch. Medicine, Baltimore, MD, 21205
J Biol Chem; 257(7):3970-3977 1982

A 110,000-dalton plasma membrane glycoprotein of mouse cells has been identified, purified, and characterized by use of a xenogeneic monoclonal antibody. The glycoprotein was a major component of the NIH/3T3 cells surface. It contained 15% of [3H]glucosamine incorporated into cell proteins and was composed of at least 16 isomorphic variants with apparent mol wt from 100,000 to 120,000 and isoelectric points between 6.5 and 8.1. The glycoprotein constituted 0.1% of total cell protein, as measured by the yield of purified protein, and there were over 10(6) antibody-binding sites/cell. Immunoprecipitation from pulse-chase labeled cells showed that the 110,000-dalton glycoprotein was initially synthesized as a 92,000-dalton microsomal polypeptide which gradually was converted to the mature surface form. The turnover time of the surface form was at least 20 hr. The antigenic determinant recognized by the monoclonal antibody was species-specific and nonpolymorphic. It was present in high concentration on most dividing murine cells in culture, whereas the concentration in normal mouse tissues differed widely. Among lymphoid tissues, antigen concentration was enriched in bone marrow as compared to spleen and thymus. Among nonlymphoid tissues, antigen concentration was 10-fold greater in kidney than in brain, liver, and skeletal muscle. The glycoprotein was purified without loss of antigenic activity by antibody affinity chromatography. The single step procedure yielded 3 mg of pure glycoprotein from 3 g of crude cell extract. The purification and characterization of this major membrane protein provide a basis for further study of its cell surface structure and function. (Author abstract) (50 Refs)

7. MODULATION BY ISLET-ACTIVATING PROTEIN OF ADENYLATE CYCLASE ACTIVITY IN C6 GLIOMA CELLS.

Katada T, Amano T, Ui M
Dept. Physiological Chemistry, Hokkaido Univ., Sapporo 060, Japan
J Biol Chem; 257(7):3739-3746 1982

The cAMP content of intact cells as well as adenylate cyclase of the membrane-rich particulate fractions was studied with C6 glioma cells that had been exposed to the culture medium supplemented with islet-activating protein (IAP), one of the pertussis toxins. Both the increase in the cellular cAMP content in response to a beta-adrenergic agonist and the stimulation of membrane adenylate cyclase by the beta-agonist and/or GTP were markedly enhanced by the IAP treatment of C6 cells, but no change was induced in affinities of the agonist (or an antagonist) or GTP for their respective sites of action (or binding). The concentration of IAP required for the half-maximal enhancement was as low as 1 pg/ml, when the time of cell exposure to the toxin was prolonged to 18 hr. No enhancement was observed for the basal cAMP content or basal enzyme activity, nor was activation of adenylate cyclase by Gpp(NH)p (or NaF) affected by IAP treatment. The Vmax value of a specific and low Km GTPase was significantly smaller in the membranes of IAP-treated cells than in those of control cells. Cholera toxin treatment of cells activated adenylate cyclase without exerting any influence on these IAP actions. Thus, IAP would appear to enhance beta-receptor-coupled stimulation of adenylate cyclase, in a manner distinct from cholera toxin, by rendering more GTP available to the GTP sites in the regulatory subunit of the receptor-enzyme system. (Author abstract) (20 Refs)

8. RESISTANCE TO PHOSPHATASE OF THIOPHOSPHORYLATED EPIDERMAL GROWTH FACTOR RECEPTOR IN A431 MEMBRANES.

Cassel D, Glaser L
Dept. Biological Chemistry, Div. Biology and Biomedical Sciences, Washington Univ. Sch. Medicine, St. Louis, MO, 63110
Proc Natl Acad Sci USA; 79(7):2231-2235 1982

Epidermal growth factor (EGF) increases the phosphorylation of its receptor and other membrane proteins, and these proteins can be rapidly dephosphorylated by membrane-bound protein phosphatase. We report that [35S]adenosine 5'-[gamma-thio]triphosphate is as effective as [gamma-32P]ATP as substrate for the EGF receptor-associated protein kinase in A431 membranes. Both the kinetics and the extent of the EGF-dependent thiophosphorylation at 0 C are similar to those obtained with [gamma-32P]ATP, provided that ATP hydrolysis by the membrane preparation is inhibited by addition of adenosine 5'-[beta,gamma-imino]triphosphate. The thiophosphorylation reaction requires Mn++ but differs from the phosphorylation reaction in the inability of Mg++ to serve as cofactor. Both EGF-dependent phosphorylated and thiophosphorylated membrane proteins yield the same two major bands of Mr 145,000-160,000 in autoradiograms of Na-DodSO4/polyacrylamide gel electrophorograms. The rate of dephosphorylation of membrane proteins that have been thiophosphorylated in the presence of EGF is dramatically slower (factors of 1/20 to 1/40) than that of the phosphorylted proteins at both 0 C and 32 C. This increased metabolic stability of the thiophosphorylated proteins will be useful for investigation of the role of phosphorylation in the biological effects of EGF. (Author abstract) (22 Refs)

9. NEW CARBOHYDRATE ANTIGEN FOUND IN LARGE GLYCOPEPTIDES OF TERATOCARCINOMA CELLS.

Ozawa M, Yonezawa S, Miyauchi T
Dept. Biochemistry, Kagoshima Univ. Scn. Medicine, 1208-1 Usuki-cho, Kagoshima 890, Japan
Biochem Biophys Res Commun; 105(2):495-501 1982

A cell surface carbohydrate antigen from murine teratocarcinoma OTT6050 was investigated and compared to surface antigens from other cancer cells and adult tissues. Glycoproteins were isolated by affinity chromatography on agarose-conjugated Dolichos biflorus agglutinin (DBA), a lectin specific for the alpha-N-acetylgalactosaminyl residue. From rabbit antiserum against DBA receptors of OTT6050, specific antibodies reacting with the large glycopeptide from F9 embryonal carcinoma cells were isolated by immuno-affinity chromatography on a column containing the Sepharose-immobilized OTT6050 glycopeptide. The antigen(s), whose antigenic determinant was assumed to be N-acetylgalactosamine based on its DBA binding activity, was designated as TC (teratocarcinoma-derived carbohydrate) antigen. Molecules carrying TC antigen from OTT6050 and from F9 migrated as glycoproteins of apparent mol wt more than 300,000 upon sodium dodecyl sulfate gel electrophoresis. Immunofluorescence using the purified anti-TC antibodies detected the TC antigen in the stem and endothelial cells of OTT6050, GRSL leukemia cells, and 2-4 cell mouse embryos. The antigen was not detected in morulae or

any of several adult tissues tested. TC antigen can be distinguished from other antigens found in embryo carcinoma cells, eg SSEA-1 and F9. Its absence from sheep and human erythrocytes also distinguishes the TC antigen from Forssman, ABH, and I antigens. TC antigen should prove to be a useful marker in embryological and oncological studies. (21 Refs)

10. ANTIGEN EXPRESSION AND IMMUNE LYSIS OF HUMAN MELANOMA CELLS: ANALYSIS WITH MONOCLONAL ANTIBODIES.

Imai K, Yachi A, Ng AK, Ferrone S
Dept. Internal Medicine, Sapporo Medical Coll., Sapporo, Japan
Protides Biol Fluid Proc Colloq; 29:883-888 1982

The complement dependent and cell dependent lysis of cultured human melanoma cells (line Colo38) sensitized with monoclonal xenoantibodies to tumor-associated antigens and to normal surface markers was investigated. The relationship between the increase in the number of antigen-antibody interactions on cell membrane and extent of immune lysis was studied. A significant difference in the ability of monoclonal antibodies to human melanoma-associated antigens, to HLA-A,B antigens, to Ia-like antigens, and to a membrane-bound glycoprotein with an apparent mol wt of 85,000 daltons to mediate cell dependent and complement dependent lysis of the Colo38 was observed. No relationship was found between the lytic efficiency of the antibodies and the level of expression of the corresponding determinants on the cell membrane. While sensitization of target cells with combinations of antibodies increased the number of antigen-antibody interactions on the cell membrane, no significant change was observed in the extent of lysis of target cells. Human complement did not affect lysis of melanoma cells, even when they were sensitized with combinations of monoclonal antibodies to the markers tested. Augmentation of HLA-A,B antigens on interferon-treated melanoma cells was associated with an increase in the extent of immune lysis mediated by the corresponding antibodies. Since the relationship between changes in antigen density and extent of immune lysis is not a general phenomenon, the conflicting results in the literature on the role of antigen density in immune lysis can be explained. (9 Refs)

11. IDENTIFICATION AND PURIFICATION OF A MR 75,000 CELL SURFACE HUMAN MELANOMA-ASSOCIATED ANTIGEN.

Heaney-Kieras J, Bystryn JC
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Cancer Res; 42(6):2310-2316 1982

Surface macromolecules on cultured human melanoma cells were radioiodinated by a lactoperoxidase method. Macromolecules shed into culture medium were collected, concentrated, and fractionated on Sepharose 6B and lentil lectin-Sepharose. Radioactivity associated with macromolecules was assayed by precipitation with trichloroacetic acid, and that associated with melanoma-associated antigens (MAA) was assayed by specific immunoprecipitation with xenogeneic anti-melanoma serum. Following the last step in purification, approx two-thirds of the acid-soluble radioactivity in the most MAA-active fraction was associated with this antigen. MAA concentration in this fraction was approx 200-fold greater than that in cells, and it contained a single labeled protein band by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. The MAA was a glycoprotein consisting of a single polypeptide chain with a mol wt of approx 75,000. This antigen was common to several but not to all melanomas. It was not detected, or was present in much decreased concentration, in 16 other unrelated allogeneic or xenogeneic normal and malignant cells. It was immunologically unrelated to serum albumin or fibronectin. These observations indicate that we have highly purified a Mr 75,000 cell surface component of human melanoma cells which appears to be a MAA. (Author abstract) (38 Refs)

12. A 94,000-DALTON GLYCOPROTEIN EXPRESSED BY HUMAN MELANOMA AND CARCINOMA CELLS.

Imai K, Wilson BS, Bigotti A, Natali PG, Ferrone S
(c/o Ferrone) Dept. Pathology and Surgery, Columbia Univ., 630 West 168th St., New York, NY, 10032
JNCI; 68(5):761-769 1982

The IgG2a monoclonal antibody (MoAb) 376.96S, secreted by a hybridoma derived from a mouse immunized with cultured human melanoma cells COLO 38, reacts with a single 94,000-dalton glycoprotein that is peripherally associated with the plasma cell membrane of cultured melanoma cells. Indirect immunofluorescence analysis with cryostat thin sections of human tissues showed that this antigen is absent from a large variety of normal tissues but is readily detectable on melanomas, nevi, and several different carcinomas. The MoAb376.96S binds with cultured melanoma and carcinoma cell lines to a similar extent and can mediate both complement-dependent and cell-dependent lysis of these cells. The 94,000-dalton glycoprotein detected by MoAb376.96S is distinct in its tissue distribution, antigenicity, and molecular profile from several structures previously identified with monoclonal antibodies that have similar molecular weights. (Author abstract) (24 Refs)

13. FIVE MELANOMA-ASSOCIATED ANTIGENS IDENTIFIED BY MONOCLONAL ANTIBODIES.

Hellstrom KE, Hellstrom I, Brown JP, Yeh MY, Woodbury RG
Fred Hutchinson Cancer Res. Center, 1124 Columbia St., Seattle, WA, 98104
Protides Biol Fluid Proc Colloq; 29:877-882 1982

Mice were immunized with human melanomas and hybridomas were established that identify cell surface antigens. Five antigens were identified by monoclonal antibodies: antigens 3.1, p97, p155, p210, and 4.2. These antigens are described with respect to antibody, molecular nature, and specificity in cultured cells and biopsies. Antigen 3.1 is thought to be a glycolipid and exhibits specificity for a small subgroup of cultured melanomas and biopsy material from melanomas and normal tissues. Antigen p97 is a 97,000 mol wt glycoprotein with specificity for approx 50% of cultured melanomas and biopsy tissue from approx 50% of biopsied melanomas and some fetal tissues. Antigen p155 is a 155,000 mol wt protein (possibly a glycoprotein) and exhibits specificity in cultured and biopsied melanomas. Antigen p210 is a 210,000 mol wt protein (possibly a glycoprotein) exhibiting specificity in approx 30% of cultured melanomas and 25% cultures of other tumors and in biopsies of melanomas, other tumors, and adult and fetal brain. Antigen 4.2 may be a glycolipid and exhibits specificity for approx 80% of cultured melanomas and a few other tumors. The implications of these findings in diagnosis and therapy of human melanomas and other tumors are discussed. (24 Refs)

14. CYTOSKELETON-ASSOCIATED SURFACE GLYCOPROTEINS OF MURINE LYMPHOCYTES.

Rungger-Brandle E, Hoessli DC
Dept. Pathology, Univ. Geneva, Geneva, Switzerland
Protides Biol Fluid Proc Colloq; 29:63-66 1982

An extract of mouse (BALB/c, Balenti 5) lymphoma cells, surface-labeled with 125I, was fractionated under isotonic conditions and in the presence of a non-ionic detergent. A cytoskeletal subcompartment of light density (SCLD) was recovered which was further fractionated on a second sucrose gradient. A distinct set of labeled surface glycoproteins (including Thy-1) were identified which are associated with the SCLD. These glycoproteins may be linked to cytoskeletal elements and represent the receptor end of transmembrane linkages, within the detergent-resistant plasma membrane matrix. (8 Refs)

15. DETERGENT EXTRACTION OF CHOLERA TOXIN AND GANGLIOSIDES FROM CULTURED CELLS AND ISOLATED MEMBRANES.

Hagmann J, Fishman PH
Friedrich Meischer-Institut, CH-4002 Basel, Switzerland
Biochim Biophys Acta; 720(2):181-187 1982

Choleragen, when bound to various cultured cells, resisted extraction by Triton X-100 under conditions which retained the cytoskeletal framework of the cells. This resistance (greater than 75% of the bound toxin) was observed in Friend erythroleukemic, mouse neuroblastoma N18 and NB41A and rat glioma C6 cells even though the different cells varied over 1,000-fold in the number of toxin receptors. The extent of extraction did not depend on whether the cells were in monolayer culture or in suspension or whether choleragen was bound at 0 or 37 C. A similar resistance to extraction was also observed in membranes isolated from toxin-treated cells. Using more drastic conditions and other non-ionic detergents, 90% of the bound choleragen was solubilized from cells and membranes. When rat glioma C6 cells, which bind only small amounts of choleragen, were incubated with the ganglioside GM1, toxin binding was increased and the bound toxin was also resistant to extraction. When these cells were incubated with [3H]Gm1, up to 70% of the cell-associated GM1 was extracted under the mild conditions. When the GM1-labeled cells were incubated with choleragen or its B (binding) component, there was a significant reduction in the solubilization of GM1. Similar results were obtained with isolated membranes. When choleragen-receptor complexes were isolated from N18 cells labeled with [3H]galactose by immunoadsorption, only labeled GM1 was specifically recovered. These results suggest that it is the choleragen-ganglioside complex that is resistant to detergent extraction. (Author abstract) (27 Refs)

16. SOLUBILIZATION OF AN ACTIVITY REGULATING C3B FUNCTION FROM RAJI CELL MEMBRANES.

Carlson P, Ruddy S, Conrad DH
Dept. Medicine and Microbiology, Medical Coll. Virginia, Richmond, VA, 23298
Immunology; 46(1):163-173 1982

A fraction of isolated Raji cell membranes solubilized with 2 M KBr which was capable of inhibiting C3-dependent rosettes was examined for its ability to inhibit the alternative pathway of complement. It was shown to decrease alternative pathway-dependent hemolysis of sheep erythrocytes and to accelerate decay of factor B from these cells. It had no effect on C2 decay, the classical pathway analog of factor B. Inhibitory activity solubilized from Raji cells was not removed by immunoadsorption with anti-factor H or anti-factor I, two well-characterized serum C3b-control proteins. It also differed in two functional respects from these proteins. Firstly, it failed to result in cleavage of a peptide bond in C3 which is characteristic of factor I; secondly, its inhibitory activity did not synergize with factor I in inhibiting the alternative pathway, unlike factor H. These results suggest that Raji cells contain a regulatory factor in their membranes for the alternative pathway of complement which is distinct from factors H and I. (Author abstract) (18 Refs)

17. UTILIZATION OF GAMMA-32P-GTP BY ECTOPROTEIN KINASE OF 3T3 AND SV40 3T3 CELLS.

Mastro AM, Sniezek MJ
Dept. Microbiology, Cell Biology, Biochemistry and Biophysics, Pennsylvania State Univ., University Park, PA, 16802
Cell Biol Int Rep; 6(4):385-392 1982

The utilization of GTP by the membrane ectoprotein kinase of 3T3 and SV40-transformed 3T3 cells (SV40 3T3) was investigated. Gamma-32P-GTP was utilized by 3T3 about 77% of that seen with ATP. The reaction was cyclic AMP and cyclic GMP-independent and required Mg ions for activity. A comparison of the phosphorylation of quiescent 3T3, serum-stimulated 3T3, and SV40 3T3 cells showed the same relative extent of phosphorylation by ATP and GTP: SV40 3T3 greater than stimulated 3T3 greater than quiescent 3T3. In each case, the amount of incorporation with GTP was less than that with ATP. Proteins from cells phosphorylated with labeled GTP or ATP were separated by polyacrylamide gel electrophoresis. Radioautography showed generally similar labeling patterns with the two nucleotides. However, quiescent 3T3 cells exhibited a greater than 165,000 dalton protein that was labeled more extensively with ATP than with GTP. This band might represent more than one protein. The equivalent band in serum-stimulated cells was labeled to the same extent with ATP or GTP, although not as pronounced as in the quiescent cells. The rest of the banding patterns were similar to those of the quiescent cells. Thus, the 3T3 and SV40 3T3 cell ectokinase is a membrane-related to cyclic AMP-independent enzyme which can utilize GTP as well as ATP to phosphorylate similar substrates. (19 Refs)

18. THE EFFECTS OF BROMOBENZENE AND CARBON TETRACHLORIDE EXPOSURE IN VITRO ON THE PHOSPHOLIPASE C ACTIVITY OF RAT LIVER CELLS.

Lamb RG, Schwertz DW
Dept. Pharmacology and Medicine, Medical Coll. Virginia, Box 217, Richmond, VA, 23298
Toxicol Appl Pharmacol; 63(2):216-229 1982

Following in vitro incubation with bromobenzene or carbon tetrachloride, intact rat hepatocytes in 1000 g cell fractions and plasma membranes exhibited a degradation of phospholipids, indicated by the conversion of membrane-bound phosphatidic acid into diacylglycerol. Bromobenzene or carbon tetrachloride treatment also caused an increase in the activity of phospholipase C. A critical factor in the cellular injury induced by these hepatotoxins may be a disruption of the cell membrane structure due to the degradation of membrane lipids by the induced phospholipase C. (29 Refs)

19. EXPRESSION OF NORMAL LYMPHOCYTES OF TWO CELL SURFACE ANTIGENS, XENCSA AND GIX, RELATED TO THE MAJOR GLYCOPROTEINS (GP70) OF MURINE LEUKEMIA VIRUSES.

Morse HC, Taylor BA, Kozak CA, Chused TM, Sharrow SO, Hartley JW, Stockert E
Lab. Microbial Immunity, Natl. Inst. Allergy and Infectious Diseases, NIH, Building 5, Room 218, Bethesda, MD, 20205
J Immunol; 128(5):2111-2115 1982

XenCSA and GIX are two cell surface antigens related to the major envelope glycoproteins (gp70) of murine leukemia viruses. The levels of expression of these gp70 determinants were assessed in 36 recombinant inbred mouse strains and selected backcrosses derived from crosses between C57BL/6 with DBA/2 and C3H/He. These two antigens segregated in backcross mice and showed a different strain distribution pattern among the recombinant inbred mice, demonstrating that XenCSA and GIX are distinct genetic markers for different endogenous gp70 sequences. It was also shown that independent sets of genes regulate the expression of XenCSA and GIX. (Author abstract) (31 Refs)

20. TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS OF ERYTHROCYTE MEMBRANES.

Rosenblum BB, Hanash SM, Yew N, Neel JV
Dept. Human Genetics, Univ. Michigan, 1137 E. Catherine St., Ann Arbor, MI, 48109
Clin Chem; 28(4,part2):925-931 1982

In an effort to maximize the amount of genetic information that can be extracted from a blood sample, we investigated the use of two-dimensional polyacrylamide-gel electrophoresis (PAGE) to resolve the protein constituents of the erythrocyte membrane. Lyophilized membranes were dissolved in various concentrations of urea, NP-40 detergent, and mercaptoethanol and subjected to two-dimensional PAGE by a modification of the O'Farrell procedure, with use of the ISO-DALT apparatus. More than 600 spots were visible in silver-stained gels under conditions that excluded specific cytoskeleton protein components, including spectrin and actin. The reproducibility of the pattern depended highly on the precise composition of the solubilization mixture. Poor resolution was observed in the presence of actin and other proteins of high molecular mass (spectrin bands 1 and 2) when we used high urea concentrations that solubilized the entire erythrocyte membrane. The large number of polypeptides observed could not be attributed to proteolysis, because addition of proteolytic inhibitors to the membrane wash solutions did not alter the pattern on the gel. The pattern also did not appear to include erythrocyte cytosol proteins because, except for globin, none of five purified erythrocyte lysate proteins was visible in the erythrocyte membrane gels. We conclude that two-dimensional electrophoresis provides a powerful tool for the study of non-cytoskeletal erythrocyte membrane proteins. (Author abstract) (24 Refs)

21. HUMAN HEAT SHOCK GENE EXPRESSION AND THE MODULATION OF PLASMA MEMBRANE NA+,K+-ATPASE ACTIVITY.

Burdon H, Cutmore CM
Dept. Biochemistry, Univ. Glasgow, Glasgow G12 8QQ, Scotland
FEBS Lett; 140(1):45-48 1982

Evidence is presented to suggest heat shock protein (HSP) gene expression involvement in the modulation of Na+,K+-ATPase activity in HeLa cells exposed to temperature elevation. It is suggested that phosphorylation of Na+,K+-ATPase could lead to its inactivation and its reversal might require the direct association of an HSP or the participation of an HSP in a dephosphorylation reaction. These findings bear upon the use of hyperthermia in human cancer treatment. (26 Refs)

22. THE LIPID NATURE OF A TUMOUR-ASSOCIATED AUTOANTIGEN FROM A CHEMICALLY INDUCED RAT HEPATOMA.

Lando P, Berzins K, Perlmann P
Dept. Immunology, Univ. Stockholm, S-106 91 Stockholm, Sweden
Scand J Immunol; 15(2):187-193 1982

The target antigens from the complement-dependent cytotoxic antibodies in D23 tumor-bearer serum from rats carrying the chemically induced D23 hepatoma were extractable from extranuclear membranes of tumors with chloroform/methanol. The antigenic activity, measured by inhibition of D23 TBS cytotoxicity against D23 cells, was recovered in the phospholipid-containing fraction after silica gel chromatography. Preparative thin-layer chromatography (in chloroform/methanol/0.25% CaCl2) revealed that the antigenic activity migrated similarly to phosphatidylethanolamine and cardiolipin, although these phospholipids did not cause the inhibition seen with the antigenic fractions. Phospholipase A2 digestion of the phospholipid fraction did not affect the antigenic activity nor did it alter the mobility of the antigen when analyzed with thin-layer chromatography. From these results it was concluded that the antigen extracted from D23 hepatoma may be of phospholipid nature, although its molecular identity remains to be established. (Author abstract) (25 Refs)

MORPHOLOGICAL STUDIES

23. CORRELATIONS BETWEEN CELL SURFACE PROTEASE ACTIVITIES AND ABNORMALITIES OF OCCLUDENS JUNCTIONS IN RAT BLADDER CARCINOMA IN VITRO.

Pauli BU, Weinstein RS
Dept. Pathology, Rush-Presbyterian-St. Luke's Medical Center, 1753 West Congress Parkway, Chicago, IL, 60612
Cancer Res; 42(6):2289-2297 1982

Microenvironmental alterations, ie, proteolytic enzymes, may play a causative role in abnormalities of zonulae occludentes. To test this hypothesis, we compared in vitro the ultrastructure of three carcinoma cell lines which were derived from N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide-induced tumors of the rat urinary bladder. One of these lines had a high cell surface protease activity; the other two lines exhibited relatively low activities. Quantitative electron microscopy data revealed differences in configuration and distribution of zonula occludens-intramembrane fibrils among these cell lines, as indicated by means and standard deviations of zonulae occludens widths, and numbers of intramembrane fibrils. Although the total length of the intramembranous fibrils per square micrometer of occludens junction area was not statistically different in the three lines, junctional morphology varied greatly. Thus, carcinoma cells with high surface protease activities are able to synthesize near-normal amounts of intramembrane fibrils but are unable to assemble normal zonulae occludentes. This indicates that alterations in zonula occludens morphology, which have been induced by exogenous proteolytic enzymes, are identical

to those observed in a cell line with high cell surface protease activity. (Author abstract) (42 Refs)

24. LAMELLAR BODIES IN BENIGN AND MALIGNANT SCHWANNOMAS.

Hwang WS, Benediktsson H
Dept. Pathology, Health Sciences Center, Winnipeg, Manitoba, Canada
Acta Pathol Microbiol Scand [A]; 90(2):89-93 1982

A light and electron microscopic study was done on seven cases of schwannomas, four of which were benign and three were malignant. Spherical or oval cytoplasmic inclusion bodies, one to three microns in diameter, were detected in all cases. Three inclusions were demonstrable by Luxol fast blue stain. Ultrastructurally, the inclusions appeared as whorls of cell membranes reminiscent of myelin sheath. These lamellar bodies probably reflect the peculiar membrane characteristics of Schwann cells, and they provide a useful marker for recognition of benign or malignant schwannomas. (Author abstract) (8 Refs)

MEMBRANE RECEPTOR/ BINDING SITES

25. ANTI-ACETYLCHOLINE RECEPTOR ANTIBODY SYNTHESIS BY CULTURED LYMPHOCYTES IN MYASTHENIA GRAVIS: THYMIC AND PERIPHERAL BLOOD CELL INTERACTIONS.

Newsom-Davis J, Willcox N, Scadding G, Calder L, Vincent A, Appel SH, Kelly RB, Tindall RS, Koriyama K
Dept. Neurological Science, Royal Free Hosp. Sch. Medicine, London NW3 2QG, England
Ann NY Acad Sci; 377:393-402 1981

Thymus lymphocytes from myasthenia gravis (MG) patients produced anti-acetylcholine receptor (AChR) antibody in culture. Thymic cells from 10 thymoma patients with MG showed no evidence of anti-AChR production. Peripheral blood lymphocytes (PBL) from MG patients, including one of three thymoma patients, also produced anti-AChR in culture. Coculture of autologous thymic lymphocytes and PBL indicated that thymus cells can enhance anti-AChR production by PBL. Characteristics of the antibody production suggest that both antigen presenting cells and AChR-specific helper T-cells may be involved in mediating the enhancing effects of autologous thymic cells on the production of anti-AChR by PBL. (22 Refs)

26. DEVELOPMENT OF CHEMOTAXIS AND FORMYL PEPTIDE BINDING IN HUMAN PROMYELOCYTIC LEUKEMIA CELL LINE (HL60).

Wood PA, Douglas SA
Div. Allergy and Immunology, Joseph Stokes, Jr., Res. Inst., Children's Hosp. Philadelphia, Philadelphia, PA, 19104
Proc Soc Exp Biol Med; 169(4):421-426 1982

Uninduced HL60 cells do not exhibit random migration or chemotaxis. HL60 cells induced with dimethyl sulfoxide undergo myeloid differentiation and develop chemotactic responsiveness to formylated peptides, pepstatin, zymosan activated serum, and bacterial supernatants, but do not respond to other peptides. These results, and the kinetics of the appearance of specific (3H)F-methionine-leucine-phenylalanine binding support the role of specific membrane receptors in chemotaxis. (22 Refs)

27. INTERACTION OF PARTICLE-BOUND [125I]C3B, THE THIRD COMPONENT OF COMPLEMENT, WITH SPECIFIC RECEPTORS ON HUMAN B-LYMPHOBLASTOID CELLS (RAJI).

Frade R, Strominger J
Institut de Cancerologie et d'Iummogenetique, 16 avenue Paul-Vaillant Couturier, 94800 Villejuif, France
Immunol Lett; 3(3):173-178 1981

The interaction between particle-bound C3b and specific C3b receptors present on human B-lymphoblastoid cells was studied, taking advantage of the high density of the complex formed between C3b receptors on B-lymphoblastoid cells and C3b coated to zymosan particles. The process had an apparent dissociation constant equal to 10(-7) M. Results indicate that C3b associated to particles has a higher affinity for specific C3b receptors than soluble C3b. Analysis of of the dissociation rate of particle-bound C3b-C3b receptor complexes suggested that a cooperative effect was induced at the cell surface by particle-bound C3b but not by soluble C3b. Possible explanations of these results are discussed. (25 Refs)

28. STUDIES OF THE TRANSFERRIN RECEPTOR ON BOTH HUMAN RETICULOCYTES AND NUCLEATED HUMAN CELLS IN CULTURE. COMPARISON OF FACTORS REGULATING RECEPTOR DENSITY.

Frazier JL, Caskey JH, Yoffe M, Seligman PA
Div. Hematology/Oncology, Dept. Medicine, Univ. Colorado Sch. Medicine, Denver, CO, 80262
J Clin Invest; 69(4):853-865 1982

The transferrin receptor, present on reticulocytes and nucleated cells in tissue culture, has been measured with both immunoassay techniques and transferrin binding studies. The total cellular immunoreactive receptor is rapidly lost from erythrocytes during the process of reticulocyte maturation. This event parallels the loss of cell surface transferrin binding sites and RNA content, and correlates with previous studies that have measured the decline in hemoglobin synthesis. Nonhemoglobin-producing normal human fibroblasts, which appear to have a much lower iron requirement than reticulocytes, contain similar numbers of immunoreactive receptors per cell when in an active state of proliferation. Although receptor density on fibroblasts is directly related to cell proliferation, our studies demonstrate that nonproliferating fibroblasts still retain significant numbers of immunoreactive receptors and transferrin binding sites. Since additional studies indicate that proliferating cells have increased iron uptake, a simple hypothesis would predict that the parallel increase in transferrin binding sites and total cellular immunoreactive receptor associated with proliferation is related to an increased cellular iron requirement. However, the number of immunoreactive receptor molecules and transferrin binding sites is not changed when cells are grown in iron-deficient media, or in media with added transferrin-iron. This result and the lack of marked differences in receptor number on both hemoglobin-producing and nonhemoglobin-producing cells indicate that other factors besides receptor density play major roles in the regulation of cellular iron uptake, retention, and loss. (50 Refs)

29. INHIBITION OF EPIDERMAL GROWTH FACTOR BINDING TO MOUSE CULTURED CELLS BY FIBROBLAST-DERIVED GROWTH FACTOR. EVIDENCE FOR AN INDIRECT MECHANISM.

Rozengurt E, Collins M, Brown KD, Pettican P
Imperial Cancer Res. Fund, Lincoln's Inn Fields, London WC2A 3PX, England
J Biol Chem; 257(7):3680-3686 1982

Fibroblast-derived growth factor (FDGF), a basic, heat- and acid-stable polypeptide partially purified from the serum-free conditioned medium of BHK cells transformed by simian virus 40, is a potent mitogen for Swiss 3T3 cells and causes a marked reduction in 125I-labeled epidermal growth factor (125I-EGF) binding to these cells. The activity which inhibits EGF binding co-elutes with the growth-stimulating activity after gel filtration, ion exchange chromatography, and sodium dodecyl sulfate polyacrylamide gel electrophoresis. Both cellular responses are elicited by the same range of FDGF concentration in several murine cell types. The inhibition of EGF binding is rapid and results from a decrease in the apparent affinity of cellular receptors for 125I-EGF. FDGF does not affect the rate of cell-mediated 125I-EGF degradation. Several lines of evidence suggest that FDGF does not bind directly to EGF receptors. First, the effect of FDGF is dependent on the temperature of the assay; furthermore, treatment of cells with EGF results in loss of EGF receptors while exposure to FDGF from up to 24 hr does not induce 'down-regulation' of EGF receptors. Further, in A431 cells which display a large number of specific EGF receptors, 125I-EGF binding is not sensitive to FDGF. Finally, the effect of FDGF on 125I-EGF binding is not observed with isolated plasma membranes. Taken together, these findings suggest that FDGF binds to sites which are separate from EGF receptors. The results show a novel mechanism whereby a growth-promoting factor produced by a tumor cell line can rapidly modulate the affinity of the cellular receptors from EGF in an indirect manner. (Author abstract) (46 Refs)

30. ALTERED EPIDERMAL GROWTH FACTOR (EGF)-STIMULATED PROTEIN KINASE ACTIVITY IN VARIANT A431 CELLS WITH ALTERED GROWTH RESPONSES TO EGF.

Buss JE, Kudlow JE, Lazar CS, Gill GN
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Proc Natl Acad Sci USA; 79(8,part1):2574-2578 1982

To determine the role of epidermal growth factor (EGF)-stimulated protein kinase in the biological effects caused by EGF, tyrosine-specific kinase activity has been quantitated in A431 human epidermoid carcinoma cells and six variant cell lines. Because EGF inhibited proliferation of A431 cells, variants resistant to this inhibition were selected by treatment with mutagen and maintenance for 1 month in 0.1 uM EGF. After cloning and growth for 6-20 generations without EGF, the resistance of the variants to the growth-inhibitory effect of EGF was confirmed. Whereas EGF increased cellular phosphotyrosine content approx 10-fold in parental A431 cells, EGF caused smaller or undetectable increases in the six variant cell lines. Solubilized membranes from the six variants displayed diminished EGF-stimulated phosphorylation of the EGF receptor and of antibodies to p60src (the product of the Rous sarcoma virus transforming gene), which act as an exogenous substrate. The decrease in EGF-stimulated tyrosine-specific protein kinase activity varied from approx 40% (Clone 16) to approx 8% (Clone 18) of parental A431 activity. Phosphorylated EGF receptors from parental and variant cells migrated identically on sodium dodecyl sulfate/polyacrylamide gels. The number of EGF receptors in variant cells decreased in parallel with EGF-stimulated protein kinase activity, so that the specific activity of EGF-stimulated protein kinase per EGF receptor remained constant in the six variant cell lines with reductions in both activities to as low as 10%. These results suggest that this tyrosine-specific protein kinase activity mediates the growth-inhibitory effect of EGF on A431 cells and that both EGF binding and kinase activities reside in the same or tightly associated molecules. (Author abstract) (30 Refs)

31. NEUROTENSIN-RECEPTOR INTERACTIONS: BINDING AND PHARMACOLOGICAL STUDIES; BIOLOGICAL RELEVANCE; STRUCTURE-ACTIVITY RELATIONSHIPS.

Kitabgi P
Institut National de la Sante et de la Recherche Medicale, INSERM U 145, Faculte de Medecine, 06034 Nice Cedex, France
Protides Biol Fluid Proc Colloq; 29:483-486 1982

Characteristics of 3H-neurotensin (NT) binding to rat brain synaptic membranes and to a human colon carcinoma cell line (HT 29) were compared and both systems were used as radioreceptor assays for NT and NT synthetic analogues. The biological relevance of the NT-receptor interaction was studied, the peptide amino acid sequence involved in the NT-receptor interaction was defined, and the role of the amino acids present in that sequence was evaluated. (8 Refs)

32. OPIOID PEPTIDES INDUCE REDUCTION OF ENKEPHALIN RECEPTORS IN CULTURED NEUROBLASTOMA CELLS.

Chang KJ, Eckel RW, Blanchard SG
Molecular Biology Dept., Wellcome Res. Laboratories, Research Triangle Park, NC, 27709
Nature; 296(5856):446-448 1982

After several hr of exposure of monolayer cultures of mouse neuroblastoma N4TG1 cells to enkephalin, the number of enkephalin receptors measured subsequently in the extensively washed membrane preparations was greatly reduced. The reduction in receptor number seemed to be selective for opioid peptides and was antagonized by a variety of opiate agonists and antagonists. The observations are discussed in relation to the phenomenon of opioid-induced desensitization. (22 Refs)

33. LECTIN-INDUCED REARRANGEMENT OF AN IMMATURE HEMATOPOIETIC CELL SURFACE MARKER.

Phelps BM, Williamson P, Schlegel RA
(c/o Williamson) Molecular and Cell Biology, Pennsylvania State Univ., University Park, PA, 16802
J Cell Physiol; 110(3):245-248 1982

The transformed murine stem cell line 416B was treated with both fluorescein-conjugated Con A (flCon A) and the dye merocyanine 540 (MC540). Binding regions for MC540 and receptors for flCon A were found to occur on the same cell surface areas. It was suggested that the observed association between liquid-like membrane domains and surface glycoproteins may be strong enough to permit reorganization of disordered lipid regions by forces affecting the proteins associated with them. It was also shown that capping of one membrane receptor resulted in the simultaneous capping of a second. It was suggested that sugar residues recognized by these two lectins are found on the same surface protein in 416B cells. If so, the two

sugar residues must be relatively distant from one another on the protein molecule, since treatment with one at a concentration sufficient to induce cross-linking does not sterically inhibit the binding of the other. Alternatively, the two lectin receptors may be on separate molecules, with their common association with membrane regions with a high affinity for MC540 accounting for the inability to dissociate them. (8 Refs)

34. PROSTAGLANDIN SYNTHESIS AND BINDING IS INCREASED IN REGRESSING NMU MAMMARY CARCINOMA.

Liu SC, Knazek RA
Lab. Pathophysiology, NCI, NIH, Bethesda, MD
Prostaglandins Leukotrienes Med; 8(3):191-198 1982

Nitrosomethylurea-induced mammary tumors of Buffalo rats were used to study the regression after oophorectomy (oox). The amounts of prostaglandin E2 (PGE2) and prostaglandin F2 alpha (PGF2a) isolated from tumor cells membranes increased during the period of tumor growth, peaking 5 days after oophorectomy. The membrane binding of both prostaglandins (PGs) was saturable and reversible, reaching equilibrium after 30 min. The membrane binding of both PGs was 3-4x above baseline values on day 4 after oox. The membrane binding of PGE2 showed low-affinity characteristics during both tumor growth and oox-induced tumor regression. The membrane binding of PGF2a showed high-affinity characteristics during both tumor growth and oox-induced tumor regression; the post-oox increase in specific PGF2a binding was attributed to an increase in the number of PGF2a receptors. The elevated rates of PGF2a synthesis and the regression-associated increase in PGF2a receptors suggest that PGF2a may play a significant role in hormone-dependent mammary tumor regression. (22 Refs)

35. CHARACTERIZATION OF THE CHOLERA TOXIN RECEPTOR ON BALB/C 3T3 CELLS AS A GANGLIOSIDE SIMILAR TO, OR IDENTICAL WITH, GANGLIOSIDE GM1. NO EVIDENCE FOR GALACTOPROTEINS WITH RECEPTOR ACTIVITY.

Critchley DR, Streuli CH, Kellie S, Ansell S, Patel B
Dept. Biochemistry, Univ. Leicester, Leicester LE1 7RH, England
Biochem J; 204(1):209-219 1982

Balb/c 3T3 cells contain a large number [(0.8-1.6) x 10(6)] of high-affinity (half-maximal binding at 0.2 nM) binding sites for cholera toxin that are resistant to proteolysis, but are quantitatively extracted with chloroform/methanol. The following evidence rigorously establishes that the receptor is a ganglioside similar to, or identical with, ganglioside GM1. (1) Labeling of ganglioside GM1 by the galactose oxidase/NaB3H4 technique on intact cells was inhibited by cholera toxin. (2) Ganglioside GM1 was specifically adsorbed from Nonidet P40 extracts of both surface- (galactose oxidase/NaB3H4 technique) and metabolically ([1-14C]palmitate) labeled cells in the presence of cholera toxin, anti-toxin and Staphylococcus aureus. (3) Ganglioside GM1 was the only ganglioside labeled when total cellular gangliosides separated on silica-gel sheets were overlayed with 125I-labeled cholera toxin, although GM3 and GD1a were the major gangliosides present. In contrast no evidence for a galactoprotein with receptor activity was obtained. Cholera toxin did not protect the terminal galactose residues of cell-surface glycoproteins from labeling by the galactose oxidase/NaB3H4 technique. No toxin-binding proteins could be identified in Nonidet P40 extracts of [35S]-methionine-labeled cells by immunochemical means. After sodium dodecyl sulfate/polyacrylamide-gel electrophoresis none of the major cellular galactoproteins identified by overlaying gels with 125I-labeled ricin were able to bind 125I-labeled cholera toxin. It is concluded that the cholera toxin receptor on Balb/c 3T3 cells is exclusively ganglioside GM1 (or a related species), and that cholera toxin can therefore be used to probe the function and organization of gangliosides in these cells as previously outlined. (Author abstract) (35 Refs)

36. SPECIFIC RECEPTORS FOR SULFONYLUREAS IN BRAIN AND IN A B-CELL TUMOR OF THE RAT.

Kaubisch N, Hammer R, Wollheim C, Renold AE, Offord RE
Boehringer Ingelheim International GMBH, 6507 Ingelheim, W. Germany
Biochem Pharmacol; 31(6):1171-1174 1982

Attempts to demonstrate receptor binding of sulfonylureas, either in the pancreas or in other organs, have failed so far, most likely because the methods chosen by the investigators were not suitable for the detection of a highly specific binding site. Although from a theoretical point of view the pancreatic beta-cell has to be considered as the ideal organ for the demonstration of receptor binding of sulfonylureas, one has to take into account the difficulty of obtaining suitable amounts of islet material. Therefore, as a preliminary approach, a crude membrane preparation from rat brain was chosen as a model. Rat brain is being used quite extensively for receptor binding studies, of course predominantly for CNS-active compounds. However, it has recently been demonstrated that other compounds, yet to be shown to function in the brain, bind specifically to membranes of the brain. (Author abstract) (7 Refs)

37. POSSIBLE COMMON MECHANISM OF MORPHOLOGICAL AND GROWTH-RELATED ALTERATIONS ACCOMPANYING NEOPLASTIC TRANSFORMATION.

Vasiliev JM, Gelfand IM
Lab. Molecular Biology and Bioorganic Chemistry, Moscow State Univ., Korpus A, Moscow, USSR
Proc Natl Acad Sci USA; 79(8,part1):2594-2597 1982

Two main groups of phenotypic alterations usually accompany neoplastic transformations of cultured fibroblastic and epithelial cells: alterations of growth control and decreased formation of cell-substrate and cell-cell contacts. We suggest that both types of alterations are due to change of cell response to clustered membrane receptors. Clustering of certain receptors by corresponding ligands possibly induces a dual set of cellular reactions: (i) activation of cell proliferation preceded by an ordered sequence of prereplicative changes and (ii) attachment-clearing reactions that are associated with anchoring of ligand-receptor complexes by cytoskeletal components and may eventually lead to internalization of these complexes. The first stages of these two subsets of dual reactions are probably identical, but later the two chains of reactions are separated; completion of clearing reaction may stop further progress of the activation of proliferation. Attachment of the cell membrane to another surface can be regarded as a variant of unfinished clearing reaction. Transformed cells may be characterized by alterations of the dual reactions; the primary change may affect either the attachment-clearing branch or the common early stages preceding the separation of branches. These alterations may lead to facilitated activation of proliferation and also to primary or secondary decrease of the ability to perform attachment-clearing reactions in response to external ligands. (Author abstract) (54 Refs)

38. SOLUBILIZATION OF EPINEPHRINE-SPECIFIC ALPHA2-ADRENERGIC RECEPTORS FROM ADRENOCORTICAL CARCINOMA.

Nambi P, Aiyar NV, Sharma RK
Dept. Biochemistry, Univ. Tennessee, Memphis, TN, 38163
FEBS Lett; 140(1):98-102 1982

Using a zwitterionic detergent, alpha-receptors were selectively solubilized from adrenocortical carcinoma 494. The isolated receptors were determined to be exclusively of the alpha2-subtype. A major function of these receptors may be the mediation of epinephrine-induced transmembrane signal via the activation of guanylate cyclase. The alpha2-adrenergic coupled guanylate cyclase system might be one of the means of transmitting the catecholamine-sensitive biological response in the adrenocortical carcinoma cell. (27 Refs)

TRANSPORT STUDIES

39. REGULATION OF AMINO ACID TRANSPORT INTO EHRLICH CELLS. EFFECT OF 3',5'-ADENOSINE MONOPHOSPHATE ON L-LEUCINE TRANSPORT.

Cuesta Casado MC, Ramos EH
Instituto de Quimica Biologica, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Argentina
Cell Mol Biol; 27(6):635-641 1981

The effect of adenosine 3',5'-cyclic monophosphate on L-leucine transport was studied regarding its regulatory action on one parameter under pleiotypic control. The metabolic processes related to biosynthesis and degradation of cAMP operate in Ehrlich cells. An increase in the intracellular content of the cyclic nucleotide partially inhibits the transport of L-leucine. The regulatory effect is maintained in all stages of tumor growth. In vitro experiments showed no effect of cAMP or Bu2cAMP on (Na+ - K+)-ATP-A activity of plasma membranes under the same experimental conditions as in in vivo experiments. (Author abstract) (20 Refs)

40. CALCIUM MOVEMENT AND MEMBRANE POTENTIAL CHANGES IN THE EARLY PHASE OF NEUTROPHIL ACTIVATION BY PHORBOL MYRISTATE ACETATE: A STUDY WITH ION-SELECTIVE ELECTRODES.

Mottola C, Romeo D
Istituto di Chimica Biologica, Universita di Trieste, 34127 Trieste, Italy
J Cell Biol; 93(1):129-134 1982

To quantitate calcium movements and membrane potential changes in stimulated neutrophils, we have measured net fluxes of Ca++ and of the lipophilic cation tetraphenyl phosphonium by a very sensitive ion-selective electrode system. Activation of neutrophils by 3 x 10(-8) M phorbol 12-myristate, 13-acetate induces a release of approx 20% of total cell calcium, with an initial lag period of less than 10 s. The Ca++ outflux is markedly reduced in ATP-depleted cells and in the presence of a calmodulin inhibitor, thereby suggesting that it occurs by activation of the ATP-driven Ca++ pump of the neutrophil plasmalemma. Activation of neutrophils also induces a transiently increased exchange of medium 45Ca with cell calcium, which is measurable a few seconds after cell exposure to the stimulant and peaks at approx 40 s. Stimulation of neutrophils after attainment of steady-state accumulation of tetraphenyl phosphonium (resting potential of-67 mV) results in a marked depolarization, with a lag period of approx 60 s. The rate and extent of depolarization are reduced by 40 and 65%, respectively, in a low Na+ medium but are not modified by an inhibitor of anion exchange across membranes. A high-K+ medium depolarizes neutrophils without either modifying their resting oxidative metabolism or impairing stimulability by the phorbol ester. Phorbol 12-myristate, which also exhibits no effect on the oxidative metabolism of neutrophils, does not induce Ca++ extrusion and membrane potential changes. The causal relationship between Ca++ mobilization, membrane potential changes and activation of neutrophil functions is discussed. (Author abstract) (49 Refs)

41. CALCIUM PERMEABILITY OF EHRLICH ASCITES TUMOUR CELL PLASMA MEMBRANE IN VIVO.

Cittadini A, Dani AM, Wolf F, Bossi D, Calviello G
Inst. General pathology, Catholic Univ., via Pineta Sacchetti 644, 00168 Roma, Italy
Biochim Biophys Acta; 686(1):27-35 1982

Passive Ca++ entry into Ehrlich ascites tumor cells has been investigated. Passive equilibration of Ca++ takes place in ascites tumor cells only under conditions of exhaustive energy depletion. The specific Ca++ ionophore A23187 does not affect Ca++ entry into ascites tumor cells under active metabolic conditions, but it increases the rate of Ca++ equilibration in ascites tumor cells in the early stages of energy depletion. The results of the present experiments lead to the conclusion that in ascites tumor cell plasma membrane Ca++ permeability is not a limiting step in the regulation of intracellular calcium content, while the energy-dependent Ca++ extrusion is the main mechanism that prevents uncontrolled intracellular Ca++ increase. The results taken together support the hypothesis that increased Ca++ influx into the cell, caused by plasma membrane alteration, is responsible for permanently elevated mitotic activity and for deranged metabolic behavior of these neoplastic cells. (Author abstract) (28 Refs)

42. SELECTIVE ENHANCEMENT OF BLEOMYCIN CYTOTOXICITY BY LOCAL ANESTHETICS.

Mizuno S, Ishida A
Dept. Antibiotics, Natl. Inst. Health, 2-10-35, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan
Biochem Biophys Res Commun; 105(2):425-431 1982

The effect of local anesthetics on the response of cultured FM3A mouse mammary carcinoma cells to bleomycin cytotoxicity was investigated. Dibucaine markedly enhanced the bleomycin cytotoxicity in a dose dependent manner.The decrease in the survival of cells treated with graded doses of bleomycin with a fixed concentration of dibucaine was dependent on the dose of bleomycin. Procaine, lidocaine, butacaine, and tetracaine also greatly potentiated the cytotoxic activity of bleomycin. Dibucaine showed the strongest potentiating activity of all 5 anesthetics tested, and the potency of each compound to enhance the cytotoxicity was correlated to its anesthetic potency. Exposure of FM3A cells to dibucaine before treatment with bleomycin produced a similar enhancement of bleomycin cytotoxicity. This effect was maintained when bleomycin was added immediately following dibucaine treatment, and decreased as the time interval between anesthetic and bleomycin treatment increased. Dibucaine did not show the enhancement effect with either adriamycin, mitomycin C, or cisplatin, indicating that the anesthetics selectively potentiate bleomycin cytotoxicity. These results may be interpreted as a membrane

phenomenon, in which the local anesthetics interact with the membrane lipids, modifying the membrane properties and resulting in an increased transport of bleomycin into the cell or a decreased efflux once it has entered the cell. (19 Refs)

43. EFFECT OF LEVORIN ON ACTIVITY OF DIFFUSION PROCESSES IN MURINE EHRLICH'S CARCINOMA CELLS.

Lishnevskaia EB, Gorozhankina IS, Tereshin IM
All-Union Res. Technological Inst. Medicinal Antibiotics and Enzymes, Leningrad, USSR
Antibiotiki; 27(2):141-144 1982

The effect of polyene antibiotic levorin on the biological transport was studied in cultured cells of murine Ehrlich's carcinoma. Incubation of tumor cells with levorin at 0.25 ug/ml inhibited the active transport of amino acids. Levorin did not affect the transport of nucleosides or the penetration of cyclophosphamide. (17 Refs) (In RUS)

44. TRANSPORT OF 6-DEOXY-D-GLUCOSE AND D-XYLOSE BY UNTRANSFORMED AND SV40-TRANSFORMED 3T3 CELLS.

Romano AH, Connell ND
Microbiology Section, Univ. Connecticut, Storrs, CT, 06268
J Cell Physiol; 111(1):83-88 1982

Serum-stimulated and SV-40-transformed 3T3 cells were observed to have similar high rates of transport of 6-deoxy-D-glucose and D-Xylose as compared to quiescent 3T3 cells. No enhancement of transport specific for transformation was found. Discrepancies in data from different laboratories on the transport of analogs of D-glucose by untransformed vs transformed 3T3 cells might be explained on the basis of differences in the growth state of the untransformed cells used in the experiments. (32 Refs)

45. FURTHER STUDIES ON THE CHARGE-RELATED ALTERATIONS OF METHOTREXATE TRANSPORT IN EHRLICH ASCITES TUMOR CELLS BY IONIC LIPOSOMES: CORRELATION WITH LIPOSOME-CELL ASSOCIATION.

Fry DW, Goldman ID
Dept. Medicine, Medical Coll. Virginia, Richmond, VA, 23298
J Membr Biol; 66(2):87-95 1982

Interaction of positively (phosphatidylcholine/stearyl amine 5:1) or negatively (phosphatidylcholine/stearic acid 5:1) charged liposomes with Ehrlich ascites tumor cells for 1-5 min increases or decreases, respectively, the bidirectional fluxes of the folic acid analog, methotrexate. These effects on influx and efflux appear to be symmetrical since the liposomes do not change the intracellular level of methotrexate at the steady state. Influx kinetics show that these alterations result from an increase or decrease in the Vmax with no change in the Kinm. These effects appear to be specific for the methotrexate-tetrahydrofolate carrier system since the transport of other compounds which utilize this carrier, aminopterin, 5-methyltetrahydrofolate, and 5-formyltetrahydrofolate, is affected similarly to methotrexate, whereas, the transport of folic acid, a compound similar in structure and charge but not significantly transported by this carrier is unaffected by liposomes. Once cells are exposed to charged liposomes, the effects on methotrexate transport cannot be reversed by washing the cells free of the extracellular liposomes. If, however, cells are exposed to liposomes of one charge, washed and then exposed to liposomes of the opposite charge, methotrexate influx is reversed to control rates. The effects of charged liposomes on methotrexate influx were not abolished by treating the cells with neuraminidase, metabolic inhibitors or lowering the temperature to 4 C. Studies on the uptake of [14C]liposomes show that these effects are not proportional to the total amount of lipid associated with the cell but result from an initial rapid liposome-cell association that is not dependent on temperature or energy metabolism not related to cell surface charge. (Author abstract) (51 Refs)

46. HEPATOBILIARY TRANSPORT OF IGA IMMUNE COMPLEXES: MOLECULAR AND CELLULAR ASPECTS.

Brown TA, Russell MW, Mestecky J
Dept. Microbiology and Inst. Dental Res., Univ. Alabama in Birmingham, Birmingham, AL, 35294
J Immunol; 128(5):2183-2186 1982

The molecular and cellular parameters of the transport of IgA immune complexes (IgA-IC) from the circulation to the bile in vivo were studied in mice. Polymeric MOPC 315 IgA was effective in the transport of 125I-DNP-HSA into the bile, whereas complexes containing monomeric IgA were not transported. The transport of IgA-IC could be blocked by excess free IgA of different antigen specificity, but not by IgG or IgM. Hepatobiliary transport did not appear to involve the galactose- or mannose-specific glycoprotein receptors of the liver. No difference in IgA-IC transport, relative to controls, could be seen in mice that had been treated with cobra venom factor or whose mononuclear phagocytic system had been blockaded with colloidal carbon. These data suggest that the hepatobiliary transport of IgA-IC proceeds by a mechanism similar to the selective transport of free polymeric IgA through the hepatocyte. (Author abstract) (30 Refs)

47. THE INTERNAL PH AND MEMBRANE POTENTIAL OF THE INSULIN-SECRETORY GRANULE.

Hutton JC
Dept. Clinical Biochemistry, Univ. Cambridge, New Addenbrooke's Hosp., Hills Road, Cambridge CB2 2QR, England
Biochem J; 204(1):171-178 1982

The membrane potential (delta psi) and the pH gradient (delta pH) across the membrane of the insulin-secretory granule were determined in studies in vitro from the uptake of the permeant anion thiol[14C]cyanate or the permeant base [14C]methylamine. Freshly prepared granules incubated in isoosmotic medium containing sucrose and low concentrations of buffer salts exhibited an acidic internal pH and a delta psi positive inside. Addition of MgATP-- under these conditions did not alter the delta pH, but produced a marked increase in the delta psi. Conversely, when a permeant anion was also included, ATP produced a marked increase in the delta pH and a lesser increment in the delta psi. NH4+ salts reduced the delta pH across granule membranes. In the presence of ATP this effect was accompanied by a reciprocal increase in the delta psi. A similar reciprocity was evident when nigericin was added together with K+ or on decreasing the medium pH, suggesting that these gradients were linked by a common electrogenic process. The effects of ATP were reversed by the protonophore carbonyl cyanide p-trifluoromethoxyphenylhydrazone, the combination of valinomycin, nigericin and K+, and by the Mg++-dependent ATPase inhibitor tributyltin. Uptakes of 14C-labeled tracer molecules were also markedly reduced by cryogenic disruption of the granule membrane or hypo-osmotic incubation condi-

tions. These results were readily interpreted within a chemiosmotic hypothesis, which proposed that the insulin granules possess an inwardly-directed electrogenic proton-translocating Mg + +-dependent ATPase with the additional postulate that the membrane has a low proton permeability. The intragranular pH was estimated as being between 5 and 6 in vivo. Such a value corresponds to optimal conditions for the crystallization of zinc-insulin hexamers. Several other functions related to chemiosmotic processes within insulin granules, however, may be envisaged. (Author abstract) (28 Refs)

48. PROTON-TRANSLOCATING MG2+-DEPENDENT ATPASE ACTIVITY IN INSULIN-SECRETORY GRANULES.

Hutton JC, Peshavaria M
Dept. Clinical Biochemistry, Univ. Cambridge, New Addenbrooke's Hosp., Hills Road, Cambridge CB2 2QR, England
Biochem J; 204(1):161-170 1982

Insulin-secretory granules isolated from a pancreatic islet-cell tumor by centrifugation on Percoll density gradients exhibited a membrane-associated MG + +-dependent ATPase activity. In granule suspensions incubated in iso-osmotic media, activity was increased two to three-fold by carbonyl cyanide p-trifluoromethoxyphenylhydrazone, the combination of valinomycin, nigericin and K2SO4 or by the addition of a detergent. Permeant anions also increased Mg + +-dependent ATPase activity under iso-osmotic conditions when combined with K + and nigericin, or NH4 +. It was deduced that a major component of the activity was coupled to the translocation of protons into the granule interior. The granule membrane appeared poorly permeable to H +, K +, NH4 + and SO4-- but permeable, in increasing order, to phosphate or acetate, Cl-, I- and SCN-. Like the proton-translocating ATPase of mammalian mitochondria the granule enzyme when membrane-bound was inhibited by up to 85% by tributyltin or NN'-dicyclohexylcarbodi-imide and was solubilized in a tributyltin-insensitive form after extraction with dichloromethane. It was clearly not a mitochondrial contaminant as evidence by the distribution of marker proteins on density gradients. Unlike mitochondrial activity it was insensitive to oligomycin, efrapeptin, atractyloside, azide and oxyanions. Its properties, however, were indistinguishable from those of the proton-translocating ATPase found in the chromaffin granules of the adrenal medulla. Moreover, insulin granules and chromaffin granules exhibited similar levels of activity. This indicated that in spite of the differences in their internal composition, granules from tissue involved in polypeptide and amine hormone secretion possess catalytic components in common. Only a minor role for the ATPase in amine transport in insulin granules was apparent. Rather, its presence here may relate to the process of secretory vesicle morphogenesis or to the exocytotic mechanism. (Author abstract) (42 Refs)

49. EXPRESS ANALYSIS OF ANTITUMOR ACTIVITY OF CHEMICAL COMPOUNDS BASED ON CRITICAL VALUES OF STABILITY OF ION HOMEOSTASIS.

Toktamysova ZS, Malenkov AG, Birzhanova NKh, Buzurbaev GG, Balmukhanov BS
Res. Inst. Biological Testing Chemical Compounds, Moscow Oblast', USSR
Khim Farm Zh; 16(2):157-162 1982

Potential use of the model of homeostatic regulation for the express testing of new antineoplastic agents was studied in cultured cells of Ehrlich ascites carcinoma. The active transport of K + ions was shown to depend upon the intracellular concentration of ATP. Inhibition of oxygen consumption was shown to cause the decrease of intracellular concentration of ATP and the complete inhibition of K + transport. It was concluded that the level of K + transport in tumor cells reflects the state of homeostatic ATP production. (14 Refs) (In RUS)

CELL ADHESION

50. ROLE OF LAMININ IN THE ATTACHMENT AND METASTASIS OF MURINE TUMOR CELLS.

Terranova VP, Liotta LA, Russo RG, Martin GR
Lab. Developmental Biology and Anomalies, Natl. Inst. Dental Res., NIH, Bethesda, MD, 20205
Cancer Res; 42(6):2265-2269 1982

We have studied the attachment of two murine metastatic cell lines and of a transformed, nonmetastatic sarcoma cell line to Type IV (basement membrane) collagen. The metastatic cells attached preferentially to Type IV collagen, whereas the nonmetastatic cells attached best to Type I collagen. Laminin increased both the rate and the number of metastatic cells attaching to Type IV collagen, while fibronectin had no effect. Antibodies to laminin prevented the attachment of metastatic cells to Type IV collagen, while antibodies to fibronectin prevented the attachment of the nonmetastatic cells. The number of pulmonary metastases which formed after iv injection of cells into C57BL mice was used to measure the metastatic propensity of these cell lines. A subpopulation of the metastatic cells selected for by their ability to attach to Type IV collagen in the presence of laminin produced more metastases than did unattached cells or cells attached with fibronectin. In addition, incubation of metastatic cells with antibody to laminin prior to injection into mice markedly reduced the number of lung metastases. These data suggest that laminin promotes the attachment of metastatic tumor cells to basement membrane during the metastatic process. (Author abstract) (31 Refs)

51. PROTEASE RESISTANCE AND CONFORMATION OF LAMININ.

Ott U, Odermatt E, Engel J, Furthmayr H, Timpl R
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Eur J Biochem; 123(1):63-72 1982

Laminin, obtained from a tumor basement membrane, was treated with neutral proteases (trypsin, chymotrypsin, elastase, subtilisin, Staphylococcus aureus protease), which all produced similar fragment patterns upon prolonged digestion. The patterns were different from those obtained for fibronectin, which showed a much higher susceptibility to proteolysis by the same enzymes. Four large fragments could be purified which accounted together for more than half of the mass of laminin. They were found to differ in size, amino acid composition, spectral properties and antigenicity. The largest fragment 1(Mr 260,000-300,000) was rich in half-cystine (120 residues/1,000) and showed a circular dichroism spectrum indicating the absence of alpha helix and beta structure. Fragment 3 (Mr = 50,000) possessed beta structure and was able to bind onto heparin-Sepharose. Fragments 2 (Mr = 50,000) and 4 (Mr = 75,000) were related structures and their relative yields depended on the protease used. They showed spectra similar to those of fragment 1. Electron microscopy revealed that fragment 1 consists of three rodlike elements (length 26 nm) connected to each other at one end. The other fragments appeared

as globules (fragment 3), short rods (fragment 2) or globules connected to a short rod (fragment 4). Data obtained from limited proteolysis indicated that fragment 1 and 4 (or 2) are in close proximity to each other in the three short arms of laminin, which in its intact form has the shape of an asymmetric cross. The long arm appeared to be readily degraded by proteases. (Author abstract) (29 Refs)

52. PURIFICATION OF GLYCOPROTEIN FROM 'CONDITIONED MEDIUM' OF SPONTANEOUSLY MALIGNANT TRANSFORMED FIBROBLAST CELLS; CELL-SPREADING EFFECT OF THIS GLYCOPROTEIN ON SO-CALLED RETICULUM CELLS CULTURED IN SERUM-FREE MEDIUM.

Adachi T, Midorikawa O
Dept. Pathology, Faculty Medicine, Kyoto Univ., Kyoto, Japan
Cell Mol Biol; 27(6):709-717 1981

Mouse fibroblast cells (m cells) and so-called reticulum cells (MR cells and SP cells) transformed into malignant cells in vitro, were transferred to a serum-free culture medium, and the morphological and functional differences of these two kinds of cells were studied. We found that the fibroblast cells produce a unique glycoprotein which has the biological function of inducing cell-spreading of so-called reticulum cells on the substratum in the serum-free culture medium. This glycoprotein was purified from the conditioned medium of the transformed fibroblast cells. The mol wt of this protein is about 230-250 x 10(3) daltons. The so-called reticulum cells themselves do not produce detectable amounts of this protein. Specific antiserum against this glycoprotein shows a single precipitation line against not only this glycoprotein, but also against serum protein. This antiserum inhibits the cell-spreading inducing activity of this glycoprotein in vitro. Immunofluorescent microscopy shows that this glycoprotein exists on the cell surface and in the cytoplasm of normal and some transformed fibroblast cells. Undifferentiated fibrosarcoma cells, however, do not produce this glycoprotein in vitro. (Author abstract) (24 Refs)

53. FIBRONECTIN.

Stasko T, De Villez RL
Div. Dermatology, Univ. Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX, 78284
Int J Dermatol; 21(2):68-74 1982

Structural, chemical, and functional aspects of fibronectin (FN) in the human body are reviewed, with emphasis on the glycoprotein's role in dermatological problems of structural or immunological origin. FN in transformed cell lines and in the skin is discussed; immunofluorescence patterns of healthy and diseased skin are shown. (25 Refs)

54. FIBRONECTIN IN HUMAN BREAST TUMORS. OPTICAL STUDIES IN VITRO AND IN VIVO ABOUT 19 CASES.

Noel P, Faucon M, Thevenin MA
Unite de Morphologie Cellulaire et Tissulaire, Centre Leon Berard, 28 rue Laennec, F 69373 Lyon Cedex 2, France
Ann Pathol; 2(1):41-48 1982

Fibronectin attracted so much interest in the last year because of the discovery of its decrease or disappearance on the cell membrane of transformed malignant cells. The aim of this work was to study the behavior of the human breast tumors to know if the progressive loss of fibronectin followed by its complete disappearance can be considered as a morphological marker correlated with the clinical passage from benignity to malignancy. The authors studied fibronectin in 19 cases of human breast tumors (9 malignant tumors, 9 benign tumors, and 1 case of the so-called human normal breast tissue) by indirect immunofluorescence on tumor biopsy sections (in vivo) and tumor-cell cultures (in vitro). The interest of fibronectin staining was based upon its decrease or disappearance in the cell membrane of malignant transformed cells; the basement membrane rupture is an important proof of the establishment of malignancy in human pathology. The results in vivo were as follows: In all the cases of fibroadenomas, the presence of fibronectin in the basement membrane of galactophores and adjacent myoepithelial cells was noted. These results were found in normal breast tissue samples. In fibrocystic disease, the above mentioned model was present but different: the fluorescent staining of the basement membrane by the fibronectin was limited in some parts of the membrane and had an irregular thickness. In malignant breast tumors such as infiltrating carcinomas, we noted in all cases the disappearance of fibronectin staining on basement membrane (9 cases); in several samples a lamellar aspect of the basement membrane was noted allowing clusters of malignant cells to invade the stroma. The results 'in vitro' emphasized both stages of intracytoplasmic fibronectin production and extracytoplasmic excretion; the results were different from those above mentioned 'in vivo'. (Author abstract) (10 Refs) (In FRE)

55. IMMUNOFLUORESCENCE LOCALIZATION OF FIBRONECTIN IN CHONDROSARCOMA CARTILAGE MATRIX.

Kimata K, Foidart JM, Pennypacker JP, Kleinman HK, Martin GR, Hewitt AT
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Cancer Res; 42(6):2384-2391 1982

In this study, we have compared the extracellular matrix components and the in vitro adhesion characteristics of normal rat epiphysial chondrocytes with those from the Swarm rat chondrosarcoma, which has many of the biochemical characteristics of normal cartilage. With the use of immunofluorescence techniques, tissue slices and chondrocytes in culture were tested for the presence of collagen Types I and II, cartilage-characteristic proteoglycan, and fibronectin. Both normal and tumor matrix contained Type II collagen and cartilage proteoglycan, but only the tumor matrix contained fibronectin. In culture, tumor-derived chondrocytes continued to accumulate fibronectin in their matrix, even after deposition of Type II collagen and proteoglycans, while normal chondrocytes did not. When the attachment characteristics of both types of chondrocytes were compared, tumor chondrocytes required fibronectin for attachment, while normal chondrocytes used another attachment factor that had been identified previously as chondronectin. These studies suggest that, although biochemically similar to normal chondrocytes, tumor chondrocytes are no longer able to express the regulatory mechanisms for fibronectin accumulation. (Author abstract) (44 Refs)

56. ISOLATION OF CLONED VARIANTS OF A RAT HEPATOMA CELL LINE WITH ALTERED ATTACHMENT TO COLLAGEN, BUT NORMAL ATTACHMENT TO FIBRONECTIN.

Briles EB, Haskew NB
Dept. Anatomy, Univ. Alabama in Birmingham, Birmingham, AL, 35294

Exp Cell Res; 138(2):436-441 1982

Cloned variants of a rat hepatoma cell line have been isolated which exhibit normal attachment and spreading behavior on fibronectin substrata, but which are defective in their ability to attach to native collagen films. These clones should be useful for identifying specific macromolecules involved in the cell-to-collagen interaction. (Author abstract) (17 Refs)

57. THE BINDING OF CELL SURFACE-ASSOCIATED ADHESIVE FACTOR (FROM RAT ASCITES HEPATOMA CELLS) TO THE CELL SURFACE.

Kurano R
First Dept. Pathology, Kumamoto Univ. Medical Sch., Kumamoto, Japan

Kumamoto Med J; 34(4):174-183 1981

Undifferentiated AH109A rat hepatoma cells, which normally are present as single cells in vivo, could be induced to aggregate when a glycoprotein adhesive factor (AF) extracted from AH136B cells was exogenously applied. Binding of AF to AH109A cells seemed to be completed within 30 min of application; aggregation was proportional to the quantity of AF applied. Cell aggregation could be induced at 22 or 37 C, but not at 4 C. The potency of AF could be blocked by exposure of the AF to 100 milliM D-mannose. Indirect immunofluorescence micrographs illustrated the pattern of distribution of AF on the surface the AH109A cells. Development of a patchy appearance (or clustering of adhesive sites) seemed to be a prerequisite for aggregation of cells. Cells fluorescence-stained at 4 C, or fixed cells, did not exhibit patch formation. (20 Refs)

58. THE SYNTHESIS OF CELL SURFACE-ASSOCIATED ADHESIVE FACTOR BY RAT ASCITES HEPATOMA CELLS AND ITS LOCALIZATION.

Kurano R
First Dept. Pathology, Kumamoto Univ. Medical Sch., Kumamoto, Japan

Kumamoto Med J; 34(4):164-173 1981

Synthesis of a glycoprotein adhesive factor (AF) by AH136B rat hepatoma cells and its localization on the cell surface by indirect immunofluorescence is described. Binding assay of 125I-labeled antibody against AF using dissociated cultured cells demonstrated resynthesis of the AF by the cells, which was inhibited in the presence of puromycin or actinomycin D. Indirect immunofluorescence localized AF on the surface of AH136B cells, but not on AH109A cells. Immunological studies showed that the adhesive component regenerated on the surface of AH136B cells may be specifically bound with the antibody (Fab fragment) against the component, which was exogenously applied to the cells, resulting in failure of aggregation. Cell adhesion seems to require the synthesis of AF on the cell surface of AH136B cells. Separation of AF from the cells was possible for AH136B cells, but could not be accomplished with AH109A cells, suggesting that the latter do not synthesize AF. (28 Refs)

MEMBRANE BIOGENESIS AND METABOLISM

59. CHANGES IN PHOSPHOLIPID POLAR HEAD GROUP TURNOVER IN SV40-TRANSFORMED HAMSTER FIBROBLASTS.

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FEBS Lett; 139(2):217-220 1982

The effect of viral transformation on turnover of phospholipid polar head groups was studied in vitro in hamster fibroblasts. Cell cultures of SVEHSVi hamster fibroblasts transformed by simian virus 40 (SV40) and cultures of nontransformed EHB hamster fibroblasts were labeled with 0.1 uCi/ml [U-14C]inositol, 0.5 uCi/ml [methyl-14C]choline, or 0.5 or 5 uCi/ml [2-14C]ethanolamine. Phospholipids from cell suspensions were separated by chromatography using chloroform/methanol solvent. Transformed cells incorporated inositol into phosphatidylinositol (PI) approx 2x as rapidly as nontransformed cells. Incorporation of ethanolamine into phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was increased by a similar amount in transformed cells. Incorporation of choline into PC and sphingomyelin (SM) was decreased in transformed cells compared with nontransformed cells. Labeling of SM was delayed compared with labeling of PC. Only 1% of labeled ethanolamine was recovered in PC, indicating that not all PE was transmethylated into PC. Results indicate that transformed cells have a more rapid turnover of phospholipid polar head groups than do nontransformed cells. The significance of the turnover is uncertain, but may be related to cellular adhesive properties. (26 Refs)

60. TURNOVER OF MITOCHONDRIAL INNER MEMBRANE PROTEINS IN HEPATOMA MONOLAYER CULTURES.

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Dept. Biochemistry, Sch. Medicine, Oregon Health Sciences Univ., Portland, OR. 97201

J Biol Chem; 257(7):3575-3580 1982

The degradation rates of inner mitochondrial membrane proteins were examined in serum-deprived hepatoma cultures. In those nonproliferating cells, the degradation of composite mitochondrial proteins was a first order process with a half-life of 34 hr. The half-lives of specific inner mitochondrial membrane polypeptides were determined by examining the 3H/35S of isolated polypeptides from cells given [3H]methionine and [35S]methionine pulses, respectively, before and after a 2-day chase period. The 33 most abundant polypeptides resolved on a bidirectional polyacrylamide gel system showed half-lives ranging from 20 to 100+ hr. The 15 polypeptides translated on mitochondrial ribosomes in the presence of inhibitory concentrations of cycloheximide also displayed heterogeneous rates of degradation (t 1/2 = 35-100+ hr). None of the isolated adenosine triphosphatase (coupling factor F1) or immunoprecipitated cytochrome c oxidase subunits were significantly turned over during the case period. Five of eight cytochrome b-c1 complex subunits, however, were turned over significantly more rapidly (t 1/2 = 39-42 hr) than the other three (t 1/2 = 94+ hr). The results demonstrate heterogenous degradation rates for inner membrane polypeptides, extending in some cases to those within the same respiratory complex. (Author abstract) (49 Refs)

61. THE RELATIONSHIP BETWEEN DIFFERENTIATION OF FRIEND ERYTHROLEUKEMIA CELLS AND SYNTHESIS OF PLASMA MEMBRANE-BOUND GLYCOPROTEINS.

Bosman GJ, Boer P, Steyn-Parve EP
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Protides Biol Fluid Proc Colloq; 29:67-70 1982

Changes that plasma membrane-bound glycoproteins go through during dimethyl sulfoxide (DMSO)-induced differentiation of Friend virus-transformed murine erythroleukemia cells (FELC) are reported. When FELC were induced with DMSO in the presence of tunicamycin, Hb synthesis was inhibited, seemingly by arresting the cells just before they became irreversibly committed to differentiation. Results suggest that changes in synthesis of plasma membrane-bound glycoproteins may play a role in the differentiation of Friend erythroleukemia cells. (11 Refs)

62. TURNOVER AND REGULATION OF NA-K-ATPASE IN HELA CELLS.

Pollack LR, Tate EH, Cook JS
Dept. Developmental Biology and Cancer, Albert Einstein Sch. Medicine, 1300 Morris Park Ave., Bronx, NY, 10461
Am J Physiol; 241(5):C173-C183 1981

The relation of cellular synthetic and degradative mechanisms (turnover) to the regulation of Na-K-ATPase in HeLa cells is reviewed. Some studies reported focus on the human HeLa cell line, a simplified model of which is illustrated. Under normal growth conditions there are 10(6) Na-K-ATPase molecules on the cell surface as estimated by specific binding with ouabain (OU). Studies with OU show that the ligand is internalized at a rate corresponding to the turnover of three sets of Na-K-ATPase enzymes/generation. The label is taken up exclusively in a particulate cell compartment identified as lysosomal. Turnover is important in the recovery of cells from glycoside intoxication; unmetabolized glycoside is released by exocytosis. 13C-Density-labeled Na-K-ATPase has been identified by specific phosphorylation of its catalytic subunit with [32P]ATP or [33P]ATP, and the rate of turnover is the same as the internalization of the OU-labeled site. There is a transit time of 4 hr from onset of synthesis of the catalytic subunit (CS) to its insertion in the surface membrane; 2,800 CS are synthesized/min/cell, and 2,100 are turned over. In response to K + starvation, cells double the number of surface transport sites in 24-30 hr. In this reversible modulation of the surface density of Na-K-ATPase the synthetic rate remains constant; the number of functional enzymes/cell is controlled by change in the rate constant for turnover. (33 Refs)

63. PLASMA MEMBRANE CHANGES OF LIVER AND MORRIS HEPATOMA INDUCED BY RETINOL IN RATS.

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Cancer Res; 42(6):2450-2456 1982

The uptake and binding of po-administered labeled all-trans-retinol was studied in vivo in rats bearing nine different types of Morris hepatomas. Radioactivity was found in acid-precipitable and acid-soluble fractions of serum, liver, and the respective tumor. In the different types of Morris hepatomas, the uptake of retinol was found to be decreased. Evidently, the decreased uptake of retinol is a common feature of Morris hepatomas and seems to be related to malignant transformation and not to increased growth. A 67% decrease in the incorporation rate of labeled L-fucose and a simultaneously increased turnover of protein-bound L-fucose were the major alterations of the fucoprotein metabolism in the plasma membrane fraction of the liver induced by high doses of retinol. Protein synthesis in retinol-treated rats was unchanged in liver, hepatoma and serum protein during the 1st hr after pulse. However, 2 hr after the L-methionine pulse, an additional increase of the incorporation rate was observed. Fluorographic analysis of the plasma membrane polypeptides revealed characteristic changes in the labeling pattern after labeling with L-fucose in vivo. This study indicates that the increased hepatic secretory activity after high doses of retinol leads to rapid turnover of incompletely fucosylated glycoproteins of the plasma membrane. The subsequent alterations in the glycosylation pattern as revealed by labeling with L-fucose in vivo are not detectable in the tumor plasma membrane. Therefore, the tumor plasma membrane is not the main target organelle of retinol. Possibly, changes in the host rather than in the tumor itself may be responsible for the anticarcinogenic effect of retinol and the retinoids. (72 Refs)

64. AUTOCATABOLISM OF SURFACE MACROMOLECULES SHED BY HUMAN MELANOMA CELLS.

Bystryn JC, Perlstein J
Dept. Dermatology, New York Univ. Sch. Medicine, 560 First Ave., New York, NY, 10016
Cancer Res; 42(6):2232-2237 1982

The fate of cell surface macromolecules released by human melanoma cells in vitro was studied. Labeled surface macromolecules released by lactoperoxidase-radioiodinated melanoma cells were incubated with unlabeled cells. It was found that some of these macromolecules were autocatabolized to acid-soluble fragments by the cells which had released them. Degradation did not occur in the absence of cells, was almost completely inhibited at 4 C, and was partially suppressed by cytochalasin B (10 ug/ml) and by some inhibitors of energy production, ie, iodoacetamide (10(-4) M) and a combination of 2-deoxyglucose (18 mg/ml) and 2,4-dinitrophenol (10(-4) M). Radioiodinated surface macromolecules were degraded much more rapidly than radioiodinated serum proteins. Thus, degradation required the presence of cells, was in part an active process, and was selective. These results suggest that one of the pathways for the turnover of surface macromolecules on tumor cells is shedding followed by autocatabolism of the shed material by the cells which they have released. (Author abstract) (33 Refs)

65. THY-1 ANTIGEN ON NORMAL AND NEOPLASTIC RAT MAMMARY TISSUES: CHANGES IN LOCATION AND AMOUNT OF ANTIGEN DURING DIFFERENTIATION OF CULTURED STEM CELLS.

Rudland PS, Warburton MJ, Monaghan P, Ritter MA
Ludwig Inst. Cancer Res. (London Branch), Royal Marsden Hosp., Sutton, Surrey, SM2 5PX, England
JNCI; 68(5):799-811 1982

With the use of immunofluorescent and immunocytochemical techniques on histologic sections of mammary glands from inbred WF rats, the thymocyte differentiation antigen (Thy-1) was identified partially on and immediately adjacent to the myoepithelial cells of ducts and alveoli. This antigen was absent from epithelial cells lining such structures. Some fibroblastic cells external to these structures also bore Thy-1. During glandular development the intensity of fluorescence due to the

binding of Thy-1 antibodies increased as the myoepithelial cells matured. Similarly, mammary adenocarcinomas induced by 7,12-dimethylbenz[a]anthracene (DMBA) and N-methyl-N-nitrosourea contained elongated, presumptive myoepithelial cells that demonstrated varying degrees of Thy-1 immunofluorescence. This phenomenon was qualitatively mimicked by cultured cell lines from normal and DMBA-induced tumors. Elongated myoepithelial-like and fibroblast-like cells possessed Thy-1, whereas the cuboidal epithelial cell lines failed to express this antigen on the cell surface. Measurement of the relative number of Thy-1 molecules per cell by antiserum absorption techniques suggested that a neoplastic stem cell line, rat mammary (Rama) 25, contained about 3 x 10(5) Thy-1 molecules per cell in a form not directly accessible at the cell surface to anti-Thy-1 antibodies; the number of cryptic Thy-1 molecules was reduced when this cell line differentiated to alveolar-like cells in culture. However, when this cell line differentiated to myoepithelial-like cells, approx 5 x 10(6) molecules per cell were exposed to anti-Thy-1 antibodies with a concomitant reduction of the cryptic pool. Morphologic maturation of elongated, myoepithelial-like cell line variants was also accompanied by increased surface Thy-1 fluorescence. Thus some of the myoepithelial cells in normal glands and tumors may arise by differentiation of epithelial stem cells and a spectrum of maturation states of the myoepithelial cell may exist as monitored by cellular morphology and surface Thy-1 expression. (Author abstract) (44 Refs)

OTHER RELATED STUDIES

66. CANCER 1980: ACHIEVEMENTS, CHALLENGES, AND PROSPECTS.

Clark RL
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Cancer; 49(9):1739-1745 1982

This review article touches on various categories of research that have been expanded or made possible predominantly by funding through the National Cancer Program of the National Cancer Institute and the American Cancer Society. Under diagnosis, categories mentioned are biological markers, chromosome banding techniques, fluorescent-activated cell sorter identification of cell surface antigens, ultrastructural studies with electron microscopy, histochemical, radiologic, ultrasonographic, thermographic, angiographic techniques, nuclear magnetic resonance imaging, radioactivity labeled compounds that attach to specific tumor cell surface receptors, and other agents that are preferentially taken up by tumor tissues. Predictive tests include human tumor stem cell assays, sister chromatid exchange assay, and hormone receptor assays. The techniques listed under therapy include hyperthermia, immunotherapy, chemotherapy, radiosensitizing compounds, and the supportive measures of hyperalimentation and other nutritional manipulations, psychological reinforcement, rehabilitative efforts, bone marrow transplants, blood component therapy, protective 'germ-free' environments, and pain control. Drug delivery systems, animal and cell culture models, and prevention of carcinogenesis are also mentioned. (Author abstract) (71 Refs)

67. RELEVANCE OF LIPIDS TO HETEROTRANSPLANTATION OF HUMAN MALIGNANCIES.

Perez RL, Mitchell JR, Lozzio BB
Dept. Medical Biology/Memorial Res. Center, Center Health Sciences, Univ. Tennessee Coll. Medicine, Knoxville, TN, 37920
Oncology; 39(3):179-184 1982

Although the transplantation of human neoplasms in immunodeficient mice is now a well-established procedure, the majority of primary malignancies cannot be successfully maintained for long periods of time in adult athymic (nude) and asplenic-athymic (lasat) mice. Various lipids such as cholesterol, cholesterol oleate, stearic and palmitic acid esters markedly depress the RES phagocytic activity and immunocompetence of mammals. In view of the immunosuppressive properties of certain lipids and in order to graft and grow as many tumors as possible, further studies into the effects of lipids on the growth of heterotransplanted human tumors is warranted. Lipids may enhance local growth and facilitate the development of metastases rarely seen in nude and lasat mice bearing xenogeneic cancer cells. Lipids may accelerate human malignant cell proliferation in mice by both depressing further the defense of host and modifying the cancer cell membrane. The relationship of lipids to the onset and progression of 'spontaneous' tumors in humans is not known. (Author abstract) (35 Refs)

68. GENETIC CONTROL OF CELL-CELL INTERACTIONS.

Katz DH
Dept. Immunology, Medical Biology Inst., 11077 North Torrey Pines Road, La Jolla, CA, 92037
Pharmacol Rev; 34(1):51-62 1982

Genetic restrictions on immunocompetent cell interactions, studies on adaptive differentiation of lymphocytes in bone marrow chimeras, orchestration of cooperating phenotypes of conventional F1 lymphocytes, and parallelisms between the cell interaction and immune response phenotypes are discussed. Recent studies bearing directly on the relationships between receptors involved in intercellular communication and the phenotype of cell-cell interactions ultimately displayed by a given lymphoid cell population are emphasized. (53 Refs)

69. THE ROLE OF INHIBITED CELL-CELL COMMUNICATION IN TERATOGENESIS.

Trosko JE, Chabg CC, Netzloff M
Dept. Pediatrics and Human Development, Coll. Human Medicine, Michigan State Univ., East Lansing, MI
Teratogenesis Carcinog Mutagen; 2(1):31-45 1982

A possible common point between the promotion phase of carcinogenesis and teratogenesis, based on inhibition of cell-to-cell communication, is theoretically discussed, and the use of in vitro assays measuring intercellular communication as screening methods for teratogens is proposed. Recent work has indicated that reduced gap or tight junctions may be involved in tumor promotion. Some abnormal cell communication may affect the control of proliferation and differentiation in adult cells, resulting in tumor promotion, and interfere with important and critical communication signals during embryonic development, resulting in teratogenesis. Assays using animal cells, based on the phenomenon of metabolic cooperation, can detect disruption of cell-to-cell communication. In vitro assays similar to these but using human cells may allow screening for teratogenic agents. (151 Refs)

70. HIGHLY PERMEABLE CONTACTS AND ELECTRICAL CHARACTERISTICS OF NORMAL LIVER TISSUE. II. STRUCTURAL MODEL AND ESTIMATION OF INDUCED MOUSE HEPATOMA CELL MEMBRANE PERMEABILITY.

Sharovskaia IuIu, Mittel'man LA, Smolianinov VV, Chailakhian LM
Interdisciplinary Res. Lab. Molecular Biology and Biological Chemistry, Moscow Univ., Moscow, USSR
Tsitologiia; 24(1):26-34 1982

The values of specific resistances of nonjunctional (Rm) and junctional (Rj) membranes of induced murine hepatoma cells were calculated by means of the syncytium theory equations and on the basis of input resistances and electronic potential spread curves. For the three-dimensional model with three contacts per cell, Rm and Rj were found to be 3,300-5,600 Ohm x square centimeter (cm2) and 2 x 10(-2) Ohm x cm2, respectively. The corresponding values for normal liver cells were 2,400-7,200 Ohm x cm2 and 6.5 x 10(-2) Ohm x cm2. (5 Refs) (In RUS)

71. INFILTRATION OF LYMPHOSARCOMA CELLS INTO HEPATOCYTE CULTURES: INHIBITION BY UNIVALENT ANTIBODIES AGAINST LIVER PLASMA MEMBRANES AND LYMPHOSARCOMA CELLS.

Middelkoop OP, Roos E, Van de Pavert IV
Div. Cell Biology, Netherlands Cancer Inst., Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
Protides Biol Fluid Proc Colloq; 29:313-316 1982

Identification of cell surface components involved in the infiltration of mouse MB 6A lymphosarcoma cells (MBLC) was studied in cultures of isolated rat hepatocytes. Antisera were raised in rabbits against contiguous face-enriched rat liver plasma membranes (LPM), against whole lymphosarcoma cells, and against sinusoidal face-enriched LPM. Infiltration of the MBLC into cultures of the isolated hepatocytes was inhibited by univalent antibodies (Fab fragments from rabbits) directed against rat liver plasma membranes (anti-LPM) and against MBLC (anti-MBLC). Anti-MBLC and an anti-LPM, raised against sinusoidal face-enriched LPM, reduced the number of infiltrating MBLC solely by inhibiting adhesion of MBLC to the exposed hepatocyte surface. However, anti-LPM raised against contiguous face-enriched LPM not only reduced adhesion to the exposed surface, but also the subsequent infiltration of the adherent cells. MB 6A cells may adhere to two different hepatocyte components, one of which may be present only at the contiguous surface. A high affinity of the latter component could explain the diffuse growth pattern of MBLC in the intact liver. (10 Refs)

72. SYNTHESIS OF ADRIAMYCIN-COUPLED POLYGLUTARALDEHYDE MICROSPHERES AND EVALUATION OF THEIR CYTOSTATIC ACTIVITY.

Tokes ZA, Rogers KE, Rembaum A
Dept. Biochemistry, Sch. Medicine, Cancer Res. Center, Univ. Southern California, 1303 North Mission Road, Los Angeles, CA, 90033
Proc Natl Acad Sci USA; 79(6,part1):2026-2030 1982

Adriamycin was coupled to polyglutaraldehyde microspheres having an av diameter of 4,500 A. The coupled microspheres remained stable during incubation with cells. Full cytostatic activity was observed when the coupled adriamycin was tested with murine or human leukemia and murine sarcoma cell lines. A 10-fold increase in sensitivity was obtained with drug-resistant human leukemia cell lines. Repeated use of the coupled microspheres in the cytostatic assays did not decrease their activity, indicating that these complexes can be recycled. The results suggest that coupled adriamycin sufficiently perturbs the plasma membrane to lead to cytostatic activity. It is proposed that this mode of drug delivery provides multiple and repetitious sites for drug-cell interactions. In addition, the drug-polymer complexes may overcome those forms of resistance that are the result of decreased drug binding at the cell surface. (Author abstract) (16 Refs)

73. ARGINASE AS AN INHIBITORY PRINCIPLE IN LIVER PLASMA MEMBRANES ARRESTING THE GROWTH OF VARIOUS MAMMALIAN CELLS IN VITRO.

Terayama H, Koji T, Kontani M, Okumoto T
Zoological Inst., Univ. Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan
Biochim Biophys Acta; 720(2):188-192 1982

Plasma membranes prepared from rat livers inhibited the in vitro growth of various mammalian cells including hepatoma cells in a concentration-dependent manner, showing an almost complete arrest of cell growth at 0.1 mg protein/ml. Some of these cells tested, ie, leukemia (L1210 and P388) and myeloma (P3-NS-1/1-Ag4-1) cells, were labile in the presence of plasma membranes (losing in viability), and CHO (Chinese hamster ovary) cells became round without detaching from the substratum. The culture medium preincubated with liver plasma membranes no longer supported the growth of hepatoma cells (AH13 and AH66F). However, the 'conditioned' medium supplemented with L-arginine, supported the growth of the cells. Moreover, the addition of L-ornithine to the cultures containing plasma membranes markedly reduced the inhibitory effect of plasma membranes. The plasms membrane preparations were found to possess considerable arginase activity. These results seem to indicate the possible involvement of arginase in the inhibition of cell growth by liver plasma membranes. (Author abstract) (22 Refs)

74. STUDIES ON MANNOSYL CARRIER FUNCTION OF RETINOL AND RETINOIC ACID IN EPITHELIAL AND MESENCHYMAL TISSUES.

De Luca LM
Differentiation Control Section, NCI, Bethesda, MD
J Am Acad Dermatol; 6(4,part2):611-619 1982

Mannosyllretinylphosphate (MRP) acts as a carrier of mannose, mostly in a direct transfer to protein, in the complex biosynthetic mechanism responsible for glycoprotein assembly in mammalian membranes. Five double bonds seem to be required for this function of MRP. In liver and tracheal tissues from vitamin A-deficient hamsters and rats, retinoic acid (RA) and retinol (ROL) were equally effective in normalizing the in vivo incorporation of mannose into glycoproteins within a short time. RA also reversibly increased the adhesive properties of a spontaneously transformed mouse dermal fibroblast cell line (3T12 cells). A free carboxyl group seemed to be required for this activity of RA. Both RA and ROL enhanced the adhesiveness of 3T12 cells, but only the cellular RA-binding protein (RA-BP) was detected, suggesting that the cellular ROL-binding protein (ROL-BP) may not be a requirement for this activity. The 3T12 cells contained a metabolite of RA (compound X), different from ROL, incorporated into a mannosylretinoidphosphate (MXP). This derivative comprised up to 40% of the total pool of 3H-mannose and 14C-RA derivatives synthesized after 48 hr of labeling. The chromatographic properties of this MXP were identical to those of MRP, but the retinoid in MXP (compound X) differed from both ROL and RA. The results suggested that ROL (in the form of

MRP) and RA (in the form of MXP) may function as mannosyl carriers in biologic membranes. (27 Refs)

75. INFLUENCE OF THE LIVER ON THE PROFILE OF CIRCULATING ANTIGENS RECOGNIZED BY ANTISERUM AGAINST HEPATOMA MEMBRANE GLYCOPROTEINS.

Baumann H, Eldredge D
Dept. Cell and Tumor Biology, Roswell Park Memorial Inst., Buffalo, NY, 14263
Cancer Res; 42(6):2398-2406 1982

Rat hepatoma tissue culture cells have retained only a few liver-specific membrane functions. To find out whether transformation of these cells led to the appearance of tumor-specific membrane glycoprotein structures, which might also be present in released form in the circulation of tumor-bearing animals, we compared immunologically the sera and ascites fluids of normal and of hepatoma-bearing rats. For structural identification, we used polyspecific antibodies against the membrane glycoproteins of the hepatoma cells. Crossed immunoelectrophoresis of the body fluids resulted in the separation of several antigens, one of which appears to be tumor specific. Immunoelectrophorectic analyses of the glycoproteins from the membranes of liver and hepatoma cells revealed many shared antigens; in one instance, we could assign the membrane associated form to major antigen detected in the serum. Characterization of the molecular size and charge of two selected antigens by two-dimensional gel electrophoresis indicated that the hepatoma cell products were not completely sialylated. In the case of the circulating, hepatoma-specific antigen, this property appears to result in hepatic clearance of the most basic ('asialo') portion of the glycoprotein species via the receptor-mediated uptake of galactose-terminated glycoproteins. The result was the accumulation of the acidic forms of the antigen in the serum and ascites fluid of tumor-bearing rats. (Author abstract) (19 Refs)

76. CHARACTERIZATION OF TRANSFORMED CELLS AND TUMORS BY PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY.

Mountford CE, Grossman G, Reid G, Fox RM
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Cancer Res; 42(6):2270-2276 1982

Cultured acute lymphoblastic leukemic cells give a well-resolved proton nuclear magnetic resonance spectrum characteristic of isolated plasma membranes. We demonstrate that the signals, in the spectrum of whole cells, arise predominantly from the plasma membrane and that cells transformed by pokeweed mitogen have membranes which are significantly less rigid than are normal human peripheral blood lymphocytes. Normal thymus, malignant thymus, and a leukemic T-cell line have been compared by proton nuclear magnetic resonance spin echo experiments, and the normal thymus was found to differ. Cells transformed by the Epstein-Barr virus can also be characterized and shown to differ from the leukemically transformed cells by spin echo experiments. Since no probe molecule was required to obtain these results, this is the first definitive evidence that the structure and fluidity of the plasma membranes change as a result of transformation of lymphocytes. Proton nuclear magnetic resonance spectroscopy can now be used to compare the effect of different mitogens on T- and B-lymphocytes as well as to monitor the effects of drugs, metals, etc, on the plasma membrane of transformed lymphocytes. (Author abstract) (29 Refs)

77. 1-BUTANOL EXTRACTION AND SUBSEQUENT RECONSTITUTION OF MEMBRANE COMPONENTS WHICH MEDIATE METASTATIC PHENOTYPE.

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Dept. Surgery, Div. Immunology and Organ Transplantation, Univ. Texas Medical Sch., Houston, TX, 77030
Cancer Res; 42(6):2126-2134 1982

The purpose of this study was to examine the effect of noncytolytic 1-butanol extraction of cell membrane components upon the metastatic potential of iv injected neoplastic cells. Butanol extraction consistently increased the number of pulmonary, but not extrapulmonary, lesions observed following challenge of (a) C3H/HeJ mice with the chemically induced fibrosarcoma MCA-F or (b) C57BL/6J mice with the spontaneous melanoma B16. Variants of these cell lines which display either a low (MCA-F parental and B16-F1) or a high (MCA-F 9.4 and B16-F10) proclivity to induce pulmonary metastases were all affected by pretreatment with butanol. Organ distribution studies using 5-[125I]iododeoxyuridine-labeled B16-F10 cells demonstrated that significantly more butanol-extracted cells were initially arrested in the lungs but that the number of extracted and control cells in the pulmonary bed equalized over the first 3 hr. During the next 7 days, butanol-extracted cells were preferentially retained in the lungs, resulting in a 3-to 4-fold increase in the number of macroscopic pulmonary foci observed 3 wk later. The enhanced initial arrest of extracted cells was not mediated by increased tumor cell homoaggregation or by tumor cell:lymphocyte heteroaggregation, nor did butanol extraction select cells with an intrinsically greater metastatic potential. However, the preferential survival of extracted cells may result from an increased resistance to natural cell-mediated cytotoxicity, since (a) pretreatment of hosts with a copolymer of polyinosinic and polycytidylic acids prior to challenge with extracted cells profoundly decreased the number of lung colonies, and (b) butanol extraction of YAC-1 lymphoma target cells decreased the ability of C57BL/6J splenocytes to effect natural cell-mediated cytotoxicity in an in vitro 51Cr release assay. Finally, reassociation of the extracted moieties with the membranes of butanol-extracted cells resulted in the constitution of the original metastatic phenotype. These results suggest that cell surface structures which mediate the successful establishment of pulmonary metastases are reversibly released by brief incubation of cells in aqueous solutions of 1-butanol. (Author abstract) (37 Refs)

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SERIES CB06 No. 82/10

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. TRANSFORMATION INDUCED BY ABELSON MURINE LEUKEMIA VIRUS INVOLVES PRODUCTION OF A POLYPEPTIDE GROWTH FACTOR.

Twardzik DR, Todaro GJ, Marquardt H, Reynolds FH, Stephenson JR
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
Science; 216(4548):894-897 1982

Rat embryo fibroblast transformed by Abelson murine leukemia virus (MuLV) produce and release a transforming growth factor (TGF). Production of this factor is correlated with a tyrosine-specific protein kinase that is functionally active and is associated with the major Abelson MuLV gene product, P120. Transformation-defective mutants of Abelson MuLV do not transform cells, do not have their virus coded transforming gene product phosphorylated in tyrosine, and do not induce TGF production. Abelson MuLV-induced TGF morphologically transforms cells in culture, competes with 125I-labeled epidermal growth factor (EGF) for binding to cell receptors, and induces phosphorylation of tyrosine acceptor sites in the

HE 20.3173/2:

160,000-dalton EGF membrane receptor. After purification to homogeneity, Abelson virus-induced TGF migrates as a single polypeptide with an apparent size of 7,400 daltons as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (Author abstract) (27 Refs)

2. AFFINITY LABELING OF THE PROTEIN KINASE ASSOCIATED WITH THE EPIDERMAL GROWTH FACTOR RECEPTOR IN MEMBRANE VESICLES FROM A431 CELLS.

Buhrow SA, Cohen S, Staros JV
(c/o Staros) Dept. Biochemistry, Vanderbilt Univ. Sch. Medicine, Nashville, TN, 37232
J Biol Chem; 257(8):4019-4022 1982

Epidermal growth factor (EGF), a mitogenic polypeptide hormone, stimulates the phosphorylation of certain endogenous proteins in membrane preparations derived from A431 cells, a human tumor cell line. Membrane vesicles prepared from A431 cells were reacted with 5'-p-fluorosulfonylbenzoyl adenosine (5'-p-FSO2BzAdo). Reaction of the vesicles with 5'-p-FSO2BzAdo results in a time-dependent inhibition of EGF-stimulable protein kinase activity which parallels an increase in incorporation into the vesicles of the 5'-p-sulfonylbenzoyl-[8-14]adenosine moiety from 5'-p-FSO2Bz[14C]Ado. The primary bands labeled have Mr = 170,000 and 150,000. Labeling of these bands by 5'-p-FSO2Bz[14C]Ado is inhibited by incubation of the membrane vesicles with adenyl-5'-yl imidodiphosphate, an ATP analog. Inactivation of the kinase with N-ethylmaleimide or by heating results in a sharply decreased labeling of the proteins with Mr = 170,000 and 150,000. Proteins of these mol wt have previously been identified in these cells as the EGF receptor and a degradation product of the receptor. These experiments provide chemical evidence that the EGF receptor and the EGF-stimulable kinase are the same protein. (Author abstract) (36 Refs)

3. LIPID COMPONENT OF MEMBRANES OF ENDOPLASMATIC RETICULUM OF RAT LIVER DURING CARCINOGENESIS INDUCED BY DIETHYLNITROSAMINE.

Berdinskikh NK, Sanina KL, Gula EP, Mel'nikova NN
R. E. Kavetskii Inst. Problem Oncology, Kiev, USSR
Vestn Akad Med Nauk SSSR; (3):53-57 1982

The effect of diethylnitrosamine (DENA) hepatocarcinogenesis on the phospholipid (PL) and cholesterol content in the liver endoplasmatic reticulum was studied in random-bred albino rats. The most pronounced increase of PL content was observed during the 1st and 20th wk of carcinogenesis. The increase of cholesterol content was detected only during the late stage of carcinogenesis. (14 Refs) (In RUS)

4. TUMOR LIPIDS AND THEIR EFFECT ON THE STRUCTURE AND FUNCTION OF CELL MEMBRANES.

Diatlovitskaia EV, Bergel'son LD
Moscow, USSR
Vestn Akad Med Nauk SSSR; (3):42-47 1982

Literature data on the relationship between lipid metabolism and tumor growth are reviewed with special emphasis on the structure and function of cell membrane. Analysis of the phospholipid (PL) composition of various cell membranes showed significant differences between the rapidly growing dedifferentiated rat and mouse hepatomas and normal liver. Lipid dedifferentiation of cell membranes was also observed in other experimental tumors. The changes of PL distribution in membranes of tumor cells were found to be associated with the disturbances of the intermembrane transport of PL. The postmicrosomal supernatant of hepatoma 27 was found to contain a specific lipid-carrying protein. This protein was absent in the cytosol of normal liver cells. In addition to the changes of PL composition, microsomes and mitochondrias from hepatoma 27 cells showed marked increase of cholesterol content, and marked increase of lipid-bound sialic acids. Modification of the lipid composition of microsomes and mitochondria of hepatoma 27 was shown to result in marked stimulation of monoamine oxidase and glucose-6-phosphatase activities. (49 Refs) (In RUS)

5. REGULATION OF CELLULAR LIPID METABOLISM BY INTERFERON AND ITS RELATIONSHIP TO INTERFERON MEDIATED ANTIVIRAL STATE (MEETING ABSTRACT)

Chandrabose K, Pottathil R, Medzihradsky JL
Wellcome Research Laboratory, Research Triangle Park, NC, 27709
Fed Proc; 41(3):415 1982

Incubation of electrophoretically pure human alpha interferon (IFN) 500-1000 U/ml with human lymphoma 1M-9 cells caused drastic reductions of unsaturated fatty acids from all the major membrane phospholipids of the cells within 30 min of incubation, as detected by gas liquid chromatography of fatty acids derived from phospholipids. When concentration of IFN was increased to 1000-10,000 U/ml, the non esterified fatty acid (NEFA) content of the treated cells increased up to 100-fold or more over the control cells. Unlike the changes observed in phospholipids the changes in NEFA are not detected until 4 hrs after IFN addition to the cells and was maximal only after 8-12 hrs. Monoclonal anti IFN antibody, and inhibitors of either cellular fatty acid cyclooxygenase or soluble superoxide dismutase incubated together with IFN prevented completely both the changes in lipid metabolism. These inhibitors also prevented the development of IFN-induced antiviral state in various human cell lines. The results suggest that fatty acid cyclooxygenase and cytoplasmic superoxide dismutase participate in specific modifications of cellular lipid metabolism and that these changes appear to be necessary events for interferon mediated programming of homologous cells. (no Refs)

6. SURFACE GLYCOPROTEINS OF MALIGNANT HUMAN LEUKOCYTES.

Gahmberg CG, Andersson LC
Dept. Biochemistry, Univ. Helsinki, Helsinki, Finland
Biochim Biophys Acta; 651(2/3):65-83 1982

The biochemistry of cell surface glycoproteins of malignant WBC is reviewed. Topics discussed include surface glycoproteins of normal hematopoietic cells, cultured cell lines for malignant WBC (Epstein-Barr virus positive and negative lymphoma cell lines, histiocytic lymphoma lines, T cell leukemia, non-T, non-B cell leukemia, hairy cell leukemia, erythroleukemic cell line K562, the HL-60 promyelocytic cell line), and surface glycoproteins from patients with leukemias and lymphomas. (138 Refs)

7. REVERTANT EXPRESSING A STRUCTURAL VARIANT OF T200 GLYCOPROTEIN.

Hyman R, Trowbridge I, Stallings V, Trotter J
Dept. Cancer Biology, Salk Inst., Box 85800, San Diego, CA, 92138
Immunogenetics; 15(4):413-520 1982

The BW5147(T200-a) mutant, a T200-mutant derived from the T200 glycoprotein-positive T-cell lymphoma BW5147, was mutagenized with gamma-irradiation. Thirteen days later 1 x 10(7) cells were stained with 13/2.3 monoclonal T200-specific antibody and fluorescein-labeled goat anti-rat immunoglobulin and sorted for the most fluorescent 0.2% of cells. After fourth sorting, a detectable antigen-positive population was seen. The antigen-positive cells were split into two groups: line 1 and 2 were the most fluorescent 0.8% and 0.1%, respectively. The two lines were grown up and resorted separately and each line was cloned by limit dilution. Two clones, BW5147 x REV(T200-a) 1.1 and BW5147 x REV (T200-a)2.5, derived from lines 1 and 2, respectively, are described. The structure of the T200 glycoprotein found on the surface of the revertant cell lines was investigated by isolation of the molecule from lysates of radioiodinated cells by immunoprecipitation with monoclonal T200-specific antibody and analysis on polyacrylamide gels. The T200 glycoproteins (mol wt 105,000) isolated from the two revertant clones were much smaller than the wild-type molecule (mol wt 200,000). Results of 21P-labeling and tryptic cleavage and peptide mapping suggested that the major portion of the revertant T200 molecule is in the region of the wild-type molecule external to the plasma membrane. The implications of the such findings are discussed. (18 Refs)

8. ALTERATIONS IN PROTEIN AND GLYCOPROTEIN COMPOSITION OF RAT EMBRYO FIBROBLASTS TRANSFORMED BY HERPES SIMPLEX VIRUS TYPE 2.

Rossowski W, Blaszczyk M, Darai G, Munk K
Inst. Virus Res., German Cancer Res. Center, D-6900 Heidelberg, W. Germany
Oncology; 39(4):222-227 1982

Total and superficially exposed plasma membrane components of tumorigenic herpes-simplex-virus(HSV)-transformed fibroblasts were studied. As a result of oncogenic HSV transformation, a significant decrease of polypeptides 230,000, 180,000, 56,000 and 43,000 daltons was found. These changes were accompanied by a significant increase in superficial exposition of several sialopeptides and two peptides of 30,000 and 15,000 daltons. It was suggested that these two peptides may represent virus-coded components of HSV-transformed fibroblasts. The present results indicate that the HSV-transformed tumorigenic cell clone meets all previously described criteria for oncogenically transformed cells. (Author abstract) (38 Refs)

9. CELL COMMUNICATION REDUCED BY CHANGES IN CELL SURFACE CARBOHYDRATES.

Smets LA, Enninga IC, Van Rooy H
Dept. Experimental Cytology, Div. Cell Biology, The Netherlands Cancer Inst., 1066 CX Amsterdam, The Netherlands
Exp Cell Res; 139(1):181-189 1982

The possibility has been investigated that alterations in the plasma membrane interfere with the formation of communicating contacts between cells in vitro. To this end, various rodent cell lines and selected variant strains were simultaneously studied for the composition of membrane-bound carbohydrates and the capacity of forming intercellular communicative junctions. In addition, a variety of somatic cell hybrids of communication-incompetent mouse L cells and several normal, untransformed or neoplastic cells were investigated for the same parameters. Cell-to-cell communication was studied by measuring the cytoplasmic exchange of labeled uridine nucleotides. Maximal intercellular communication was found between cells displaying a normal size-distribution of membrane glycopeptides. In contrast, structural alterations in these molecules, reflected either by altered size-distribution or by a failure to incorporate terminal fucose, were associated with reduced or completely impaired capacity of establishing metabolic cooperation. In somatic cell hybrids complementation by normal genomes suggested the involvement of recessive genetic lesions affecting intercellular communication through disturbances in the genetic control of surface carbohydrate synthesis. The observed correlation between changes in plasma membrane molecules associated with tumor cells and impaired formation of functional cell-cell contacts may reflect a malfunction of tumor cells in their neighboring cells or with signal molecules. (Author abstract) (23 Refs)

10. STUDIES ON A TUMOR CELL SURFACE GLYCOPROTEIN THAT GRANTS RESISTANCE TO IMMUNE DESTRUCTION (MEETING ABSTRACT)

Rosenstein Y, Celis B, Escandon E, Celis E
Instituto de Investigaciones Biomedicas, National University of Mexico, Mexico
Fed Proc; 41(3):311 1982

A 160 k dalton glycoprotein (gp160) present on the cell surface of some mouse myeloma tumor lines correlates with the ability of these cells to evade immune destruction by cytotoxic T lymphocytes (CTL) and by antibodies (Abs) plus complement (C). Gp160 interacts with the H-2K and H-2D alloantigens in such way that CTL do not recognize and interact with the tumor cells. The gp160+ myeloma cells remain susceptible to the lethal mechanism of CTL since in the presence of lectins the tumor cells are lysed by the CTL. The function of gp160 in the resistance to lysis by Abs plus C remains unclear since other cell surface molecules not related to H-2 are not masked by gp160 and bind their respective Abs in a stable way. In order to study the type of interactions of gp160 with the H-2K and H-2D alloantigens and its protective role in C-mediated lysis we have started to purify and characterize this cell surface glycoprotein. Preliminary results have shown that urea extracts rich in gp160 will inhibit C action on Ab-coated red blood cells more efficiently than extracts from cells without gp160. Using salt fractionation and gel filtration techniques we have been able to obtain preparations of gp160 with a high degree of purity which are being used for sugar and amino acid composition studies. (no Refs)

11. SUGAR CHAINS ON LECTIN-BINDING GLYCOPROTEINS FROM METASTATIC MURINE B16 MELANOMA SUBLINES (MEETING ABSTRACT)

Irimura T, Nicolson GL
The University of Texas, M. D. Anderson Hospital, Houston, TX, 77030
Fed Proc; 41(3):1159 1982

Cell surface glycoproteins of metastatic murine melanoma cells (B16-F1, -F10, -BL6, -O13) are heterogenous and their carbohydrate moieties only partially elucidated. We have studied the sugar chains of the individual glycoproteins after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The method uses a combination of chemical modification of the sugar moieties and lectin staining to gels. Wheat germ agglutinin (WGA) bound to 60-67K, 80K, 93K, 115K and several

minor components. After removal of sialic acid from the glycoproteins, WGA binding was no longer detected indicating that sialic acid is responsible for the binding. After disialization Arachis hypogaea agglutinin bound to 51K, 55K and 60-67K components, but only weakly to the 80K component; whereas Ricinus communis agglutinin-I bound almost identically to WGA. These latter lectins did not bind prior to removal of sialic acid. The results suggest that high molecular weight WGA receptors do not contain mucin-type but rather asparagine-linked complex-type sugar chains with high sialic acid contents. When the desialized non-WGA binding proteins were subjected to Smith periodate degradation, they regained the ability to bind to WGA. Standard glycoproteins with tribranched, complex-type sugar chains gave similar results suggesting the existence of such carbohydrate chains on WGA receptors. Relatively small differences in lectin binding were observed among B16 sublines with various organ colonizing capacities. (no Refs)

12. ALTERATIONS IN GLYCOCONJUGATE METABOLISM IN METASTASIZING TUMORS: A POTENTIAL SITE FOR CHEMOTHERAPEUTIC MANIPULATION (MEETING ABSTRACT)

Bernacki RJ, Dobrossy L, Korytnyk W
Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, NY
VI Meeting of the European Association for Cancer Research, October 12-15, 1981, Budapest, Hungary. The European Association for Cancer Research 275 pp., 1981.

The metastatic potential of a variety of murine tumors has been correlated with the degree of cell surface sialylation. We have observed elevations in serum sialyltransferase levels in animals with metastasizing tumors and a positive correlation between cell surface sialyltransferase, sialic acid and glycosidase activities with the implantability of B16 murine melanoma in lung. These results indicate that alteration in tumor cell surface glycoconjugates may enhance a tumor's ability to metastasize. Therefore, we have embarked on a program to develop a number of sugar analogs which may act as either potential inhibitors of cell surface sialylation or inhibitors of specific lysosomal glycosidases. 6F-Galactose was developed as an inhibitor of sialic acid attachment and 2-acetamido-1,2-dideoxy-D-arabino-hex-1-enopyranose as a potential inhibitor of lysosomal N-acetylhexosaminidase. The in vitro biological activity of these compounds will be described. These compounds or other modifers of cell surface glycoconjugate may have some activity as antitumor or antimetastatic agents. (no Refs)

13. ALTERATIONS IN SPECIFIC CARBOHYDRATE RESIDUES IN NEOPLASTIC TRANSITIONAL EPITHELIUM (IE, UROTHELIUM) OF HUMAN URINARY BLADDER (MEETING ABSTRACT)

Alroy J, Gavris V, Heaney J, Szoka F, Ucci A
Departments of Pathology and Urology, Tufts University, Boston, MA
Lab Invest; 46(1):3A 1982

The antigenic nature of bladder urothelium is determined by carbohydrate residues of the cell surface coat. Loss of three specific carbohydrate residues (ie, ABH isoantigens) in neoplastic urothelium (red cell adherence test) is associated with tumor invasion and is a predictor of the clinical course. In this study, florescein isothiocyanate (FITC)-conjugated lectins were used to detect specific carbohydrate residues in neoplastic urothelium of 16 patients and compared with urothelium of 16 patients with non-neoplastic urologic disorders. Paraformaldehyde-fixed specimens were incubated with the following lectins: Ricinus communis-1 (RCA-1), Ulex europeus-1 (UEA-1), Archis hypogaea (PNA), Glycine max (SBA), Canavalia ensiformis (Con-A), Triticum vulgare (WGA), Dolichos biflorus (DBA), and Limulus polyphemus (LPA). Additional tissues were treated with neuraminidase to cleaved sialic acid residues prior to incubation with PNA. Incubation with blocking carbohydrates served as a specific negative control. RCA-1, Con-A, WGA, and LPA bound to neoplastic and non-neoplastic urothelium in all specimens. However, binding intensity varied among different neoplasms. After treatment with neuraminidase, PNA bound to all specimens except two neoplasms. UEA-1 bound to non-neoplastic urothelium in eight patients with type H blood and to some neoplastic cells in patients with type A blood. DBA bound only to non-neoplastic urothelium in eight patients with type A blood. SBA bound to blood vessel endothelium in all specimens and to metaplastic urothelium in only two patients. (no Refs)

14. EXPRESSION OF A 90K GLYCOPROTEIN WITH MURINE TYPE C VIRUS ANTIGENIC DETERMINANTS ON THE MEMBRANE OF METHYLCHOLANTHRENE-INDUCED MASTOCYTOMA P815 (MEETING ABSTRACT)

Jacquemin PC, Van Snick J, Uyttenhove C, Boon T
No affiliation given
10th International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. International Association for Comparative Research on Leukemia and Related Diseases 187 pp., 1981.

We have obtained, by mutagenesis of P815 cells with N-methyl-N'-nitro-N-nitroso-guanidine, 'tum-' clones that are rejected by syngeneic mice. Most of the 'tum-' cells were shown to confer an immune protection against the original tumor cell (tum+) that would otherwise kill the animal. We wanted to compare the cell membrane of these tum- cells with that of the original tum+ cells, for their content in type C viral proteins. Antibodies were raised in syngeneic and allogeneic mice against the tum+ cells and against some of tum- clones. The syngeneic or allogeneic mice immunized with tum+ cells had little or no antibody against purified 125Iodine labeled Rauscher murine leukemia virus (R-MuLV) gp70 while those immunized with tum- clones showed a significant precipitation of the labeled probe. The precipitation of the R-MuLV gp70 by some of these positive sera was competed with live cells and with plasma membrane (PM) preparations. Various normal DBA/2 cells and their PM did not compete, while intact P815 tum+ and tum- cells and their PM competed significantly. However, the competition with intact tum- cells was 25 times more efficient than that obtained with the tum+ cells. Iodinated membranes of normal DBA/2 thymocytes, tum+ cells and tum- cells, were precipitated with an hyperimmune rabbit serum directed against R-MuLv gp70 and with a positive syngeneic mouse serum directed against P185 tum- variant. A 90K glycoprotein was precipitated in both cases. Monoclonal anti-gp70 antibodies showed that the 90K protein contains murine endogenous ecotropic gp70. Some gag-gene products could also be precipitated from the same preparations with specific sera. The gag-gene products also precipitated as a 90K protein. (3 Refs)

15. EFFECTS OF RADIANT TREATMENT ON THE SIALOMUCINIC CONTENT IN YOSHIDA ASCITES TUMOUR CELLS: CITOPHOTOMETRIC EVALUATION (MEETING ABSTRACT)

Ricci LB, Barni S
University of Pavia, Pavia, Italy
Med Biol Environ; 9(1):445 1981

The x-ray (400 R) action on the membrane glycoproteins, glycolipids and sialic acid residues was studied in Yochida ascites tumor cells. Some cells were pretreated with neuraminidase and then all were submitted to the Hale's reaction; the stain intensity was determined by integrating microdensitometer at a wave length of 660 nm. A significant decrease of the Hale-positivity (P 0.05) has been observed in cells submitted either to radiation or to enzyme treatment. (no Refs)

16. PURIFICATION OF A NATIVE MEMBRANE-ASSOCIATED ADENOVIRUS TUMOR ANTIGEN.

Persson H, Katze MG, Philipson L
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J Virol; 42(3):905-917 1982

A 15,000-dalton protein was purified from HeLa cells infected with adenovirus Type 2. Proteins solubilized from a membrane fraction of lytically infected cells was used as the starting material for purification. Subsequent purification steps involved lentil-lectin, phosphocellulose, hydroxyapatite, DEAE-cellulose, and aminohexyl-Sepharose chromatographies. A monospecific antiserum, raised against the purified protein, immunoprecipitated a 15,000-dalton protein encoded in early-region E1B (E1B/15K protein) of the adenovirus Type 2 DNA. Tryptic finger print analysis revealed that the purified protein was identical to the E1B/15K protein encoded in the transforming part of the viral genome. The antiserum immunoprecipitated the E1B/15K protein from a variety of viral transformed cell lines isolated from humans, rats, or hamsters. The E1B/15K protein was associated with the membrane fraction of both lytically and virus-transformed cell lines and could only be released by detergent treatment. Furthermore, an 11,000-to 12,000-dalton protein that could be precipitated with the anti-E1B/15K serum was recovered from membranes treated with trypsin or proteinase K, suggesting that a major part of the E1B/15K protein is protected in membrane vesicles. Translation of early viral mRNA in a cell-free system, supplemented with rough microsomes, showed that this protein was associated with the membrane fraction also in vitro. (Author abstract) (47 Refs)

17. CALCIUM: CALMODULIN AND CANCER.

Criss WE, Kakiuchi S
Dept. Human Nutrition and Food, Cancer Center, Sch. Human Ecology, Howard Univ., Washington, DC, 20060
Fed Proc; 41(7):2289-2291 1982

When several fast growing Morris hepatoma tissue lines are compared with normal adult liver tissue, the following observations are made: calmodulin activity is increased in the cytoplasm and decreased in the memoranes of the tumor cells. Total calcium is increased three- to fivefold in the tumors. Cyclic AMP phosphodiesterase activity is increased, whereas cyclic GMP phosphodiesterase activity is decreased. In addition, several of the fast-growing Morris hepatoma tissue lines have a new calcium-binding protein that is not observed in adult liver tissue. It is probable that the Ca++-calmodulin complex is very active in these rapidly growing tumors. (Author abstract) (21 Refs)

18. THE ROLE OF CELL SURFACE PROTEINS IN THE INDUCTION OF HISTOGENETIC DIFFERENTIATIVE RESPONSES BY SOME HUMAN BREAST TUMOURS AND HAMSTER TUMOURS IMPLANTED INTO CHICK EMBRYOS.

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Cancer Res. Unit, Univ. Newcastle upon Tyne, Newcastle upon Tyne NE1 4LP, England
Exp Cell Biol; 50(3):169-179 1982

Cells of human breast tumors and fibrocystic hyperplasia grown in culture, and three hamster tumors were implanted between the cell layers of 18-hr-old chick blastoderm. Their ability to induce histogenetic responses in the ectodermal and endodermal embryonic tissues was investigated. The surface proteins of these tumor cells were labeled by lactoperoxidase-catalyzed radioiodination. It is shown that the ability to induce the histogenetic effects may be related to the expression of 265K (K = 10(3) daltons) and 233K proteins on the surface of human tumor cells and of 115 K proteins on the hamster tumor cells. The antiproteinase, aprotinin, inhibits the induction of the histogenetic responses by and apparently also prevents the deletion of 115K proteins from the hamster tumor cells. It is therefore suggested that cell surface proteins are involved in the complex processes of interaction between embryonic and tumor cells and in the recognition by the embryonic cells of the tumor cells implanted into their midst. (Author abstract) (23 Refs)

19. HIGH ACID PHOSPHATASE ACTIVITY IN THE PLASMA MEMBRANE OF HUMAN ASTROCYTOMA (MEETING ABSTRACT)

Leis JF, Kaplan NO
University of California, San Diego, La Jolla, CA, 92093
Fed Proc; 41(3):915 1982

We report the presence of a very active acid phosphatase in the plasma membrane of the human tumor astrocytoma. The enzyme showed a broad pH optima in the acid range with a maximum at pH 5.3. Substrate specificity studies show that the acid phosphatase hydrolyzes 10 mM p-nitrophenyl phosphate preferentially over other known phosphatase substrates such as B-glycerophosphate, O-phosphoryl choline and 5'-AMP. The enzymatic activity is inhibited by 10 mM fluoride (94%) and 5 mM Zn++ (42%) but not by inhibitors of other known acid phosphatases; tartrate, ouabain, oligomycin, pyridoxine HCl or mercurial reagents. Activity is stimulated 30-40% by 0.2% Triton X-100 presumably by exposing occluded sites. Triton X-100 (0.5%) has been used to solubilize the phosphatase activity and partial purification has been achieved by conventional chromatographic techniques. On a Sepharose 6-B column the enzyme shows an apparent molecular weight greater than 500,000 probably due to aggregation. The enzyme, unlike alkaline phosphatase, shows no divalent cation requirements. Recent experiments indicate that this acid phosphatase activity hydrolyzes phosphotyrosine but not phosphoserine or phosphothreonine and its role as a tyrosine phosphoprotein phosphatase is being investigated. (no Refs)

20. ALTERATIONS IN DS EXPRESSION IN SJL LYMPHOMAS (MEETING ABSTRACT)

Dyer CA, Lightbody J, Lerman SP, Kuhn M, Beisel KW
Wayne State University School of Medicine, Detroit, MI, 48201
Fed Proc; 41(3):681 1982

Variations in H-2Ks and H-2Ds antigenic expression in transplantable lymphomas (reticulum cell sarcomas) of SJL mice have been examined. Lymphomas were freed of contaminating host cells by growth in (C57BL/10 x SJL/J)F1 mice and treatment with appropriate antisera and complement. A panel of both H-2 alloantisera and monoclonal antibodies were used in immunochemical and serological assays. The H-2Ks glycoprotein was detected in all tumors by the microcytotoxicity dy exclusion assay and by immunoprecipitation using protein A. H-2Ds glycoprotein was serologically undetectable in the long term transplantable tumors, SJL/SJL and RCS5. In RCS5, the monoclonal anti-Ds precipitated a protein which was not bound by the lentil lectin column. Shorter term transplantable tumors RCSW1, RCSW8, and RCSW20 have lost the Ds glycoprotein by the dye exclusion assay. However, this Ds glycoprotein was detected immunochemically in all three tumors. We are hypothesizing that the loss of the Ds molecule, which seems to correlate with increased tumorigenicity, may be attributed to the inability of the molecule to be inserted into the cell membrane. Although the Ds protein appears to be synthesized, it probably remains in nonglycosylated form, preventing its insertion into the membrane. (no Refs)

21. EXPRESSION OF IA-LIKE ANTIGENS ON HUMAN T LYMPHOCYTES, ANALYSIS OF T LYMPHOCYTES FROM CANCER PATIENTS WITH A PANEL OF IA-LIKE MONOCLONAL ANTIBODIES (MEETING ABSTRACT)

Indiveri F, Pierri I, Rogna S, Veronese S, Grifoni V, Russo C, Ferrone S
Clinica Medica RR, ISMI, Universita di Genova, Genova, Italy
9th International RES Congress, February 7-12, 1982, Davos, Switzerland. American Reticuloendothelial Society 68 pp., 1982.

The expression of Ia-like antigens on the membrane of activated human T lymphocytes is related to the capacity of such a cells' subsets to cooperate with other lymphocyte subclasses during the immune reaction. In the present research we analyzed the expression of Ia-like antigens on T lymphocytes from 27 cancer patients, either in resting conditions or upon activation by PHA (phytohemagglutinin) in vitro. For this purpose a panel of Ia-like monoclonal antibodies (MoAb) was used in an indirect rosette microassay, Moreover we studied the capacity of non T or PHA activated T lymphocytes from cancer patients in stimulating either allogeneic or autologous cells in mixed lymphocyte cultures (MLC). Only 5 out the 27 patients had more than 10% resting T cells that reacted with the Ia-like MoAbs; upon PHA activation, the lymphocytes from 9 patients expressed Ia-like antigens. The Ia-like positive cells did not always react with the entire panel of MoAbs, showing that those MoAbs may be able to identify different functional subsets of Ia-like antigens. The MLC showed the absence of the autologous reaction, either induced by non T or by PHA activated T lymphocytes, while the allogeneic one was quite normal. In the present paper will be discussed the significance of the impairment in expressing Ia-like antigens by PHA stimulated T lymphocytes from cancer patients and the significance of their defect in MLC. (no Refs)

22. A MONOCLONAL ANTIBODY USEFUL FOR THE DIFFERENTIAL DIAGNOSIS BETWEEN MALIGNANT LYMPHOMA AND NONHEMATOPOIETIC NEOPLASMS (MEETING ABSTRACT)

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City of Hope National Medical Center, Duarte, CA
Lab Invest; 46(1):8A 1982

The distinction between non-Hodgkin's lymphoma and undifferentiated nonlymphoid neoplasms may pose difficult problems even to experienced surgical pathologists. Adjunct studies such as electron microscopy and/or immunologic methods for the demonstration of cell surface or cytoplasmic globulins do not always resolve the diagnostic dilemma. A monoclonal antibody was raised against a human T cell leukemia line by conventional hybridoma techniques and designated as T29/33 and has been previously reported to recognize a major cell surface glycoprotein restricted to hematopoietic cells. This antibody was tested as a possible tool to distinguish non-Hodgkin's lymphomas from nonhematopoietic neoplasms. Forty lymphomas and 110 nonhematopoietic neoplasms of various types were studied by indirect immunofluorescent and indirect immunoperoxidase on cryostat frozen sections. Intense staining was observed on the cell membranes of all lymphomas. All of the control tumors gave negative results, but nonneoplastic lymphocytes in or around tumor cells stained strongly, thereby acting as an internal control. (no Refs)

23. DIRECT MODIFICATION OF THE MEMBRANE ADENYLATE CYCLASE SYSTEM BY ISLET-ACTIVATING PROTEIN DUE TO ADP-RIBOSYLATION OF A MEMBRANE PROTEIN.

Katada T, Ui M
(c/o Ui) Dept. Physiological Chemistry, Hokkaido Univ., Sapporo 060, Japan
Proc Natl Acad Sci USA; 79(10,part1):3129-3133 1982

GTP and isoproterenol activation of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing) EC 4.6.1.1] in washed membranes prepared from C6 glioma cells was enhanced by incubation with islet-activating protein, one of the pertussis toxins, if the incubation mixture was supplemented with NAD and ATP. The action of the protein was observed immediately after its addition and increased progressively in magnitude as the protein concentration or the incubation time increased. There was simultaneous incorporation of radioactivity from the ADP-ribose moiety of variously labeled NAD into the membrane protein with a mol wt of 41,000. We conclude that islet-activating protein enhances receptor-mediated GTP-induced activation of membrane adenylate cyclase as a result of ADP-ribosylation of a membrane protein, probably one of the components of the receptor-adenylate cyclase system. (Author abstract) (21 Refs)

MORPHOLOGICAL STUDIES

24. ULTRASTRUCTURAL LOCALIZATION OF INTERFERON RECEPTORS ON THE SURFACES OF CULTURED CELLS AND ERYTHROCYTES.

Kushnaryov VM, Sedmak JJ, Bendler JW, Grossberg SE
Dept. Microbiology, Medical Coll. Wisconsin, Milwaukee, WI, 53226
Infect Immun; 36(2):811-821 1982

The binding sites for interferon (IFN) on the limiting cell membranes of human and mouse fibroblasts and erythrocytes were revealed by an indirect immunoferritin technique. Mouse IFN-beta and human IFN-beta of high specific activity were used with the corresponding purified antibodies. Species-specific IFN binding was demonstrated by ferritin deposition on human erythrocytes and fibroblast membranes treated with mouse IFN, and on mouse erythrocytes and fibroblast membranes treated with mouse IFN, but not on human erythrocytes or fibroblast membranes treated with mouse IFN. IFN binding sites on fibroblasts were located on regions of membranes between microvilli, whereas diphtheria toxin receptors were demonstrated mainly on microvilli. IFN binding altered the membranes of human carcinoma KB cells as evidenced by the reduced toxicity of diphtheria toxin after IFN treatment. This reduced toxicity correlated with a decrease in the quantity of receptors for diphtheria toxin on the cell membrane. Thus, the species-specific binding of IFN appears to depend on membrane receptors in discrete regions of the limiting membrane which are present not only on functionally responsive fibroblasts but also on erythrocytes. (Author abstract) (52 Refs)

25. ROLE OF ACTIN IN THE INTAKE OF FOREIGN SUBSTANCES BY LEUKOCYTES: BIOCHEMICAL MECHANISM OF PHAGOCYTOSIS.

Ueda T, Shibata N, Yoshitake J
Third Div., Osaka Adult Disease Center Inst., Osaka, Japan
Nippon Rinsho; 39(8):2709-2716 1981

Phagocytosis of foreign matters by a leukocyte occurs by approaching the foreign matter with pseudopodium, which then contacts, surrounds, and finally phagocytes the matter into the cell. During the phagocytic activity, contractile wane can be seen, suggesting the presence of contractile protein, from which actomyosin has been extracted. Thus, pseudopodium is moved by the reaction of myosin and actin, the latter is contained much more than myosin in the cell. When phagocytosis is observed by fluorescent microscopy, high fluorescent density is surrounding the foreign matter, and becomes low after intake of the foreign matter into the cell, suggesting dynamic changes in the cell. When observed by electron microscopy, high density of the microfilament was seen on the side of the pseudopodium directly in contact with the foreign matter, confirming the above observation. Four factors are important in phagocytosis: (1) surface tension of the cell membrane, (2) adhesiveness, (3) fluidity of protoplasm, and (4) energy for the formation of pseudopodium. Flowing of protoplasm is done by the contractile protein, in which actin plays an important role. Thus action's roles are creation of tension by reacting with myosin, and as cytoskeleton to maintain cell shape. (16 Refs) (In JPN)

26. CYTOPLASMIC INCLUSIONS IN LYMPHOCYTES OF CHRONIC LYMPHOCYTIC LEUKAEMIA. A REPORT OF 10 CASES.

Ralfkiaer E, Hou-Jensen K, Geisler C, Plesner T, Henschel A, Hansen MM
Dept. Pathology, Finsen Inst., DK-2100 Copenhagen, Denmark
Virchows Arch [Pathol Anat]; 395(2):227-236 1982

Peripheral blood from 90 CLL patients was examined by light- and electron-microscopy for the occurrence of crystalline inclusions in lymphocytes. Inclusions were demonstrated in 10 patients (11%). In these patients the inclusions were present in 5-45% of peripheral blood lymphocytes. In the light microscope the inclusions appeared as rectangular, unstained structures in May-Grunewald Giemsa and PAS stains. In the electron microscope the inclusions appeared as intracytoplasmic, completely partially membrane-bound bodies, which were often associated with dilated profiles of rough endoplasmic reticulum. The ultrastructure of the inclusions was granular. In immunofluorescence staining the inclusions were found to contain immunoglobulin of the same type and class as the surface membrane-bound immunoglobulin of the neoplastic lymphocytes, most frequently IgM-lambda. The lymphocytes of one case with kappa light chains at the cell surface membrane contained inclusions of the same ultrastructural morphology as those of the other cases with lambda light chains. The presence of inclusions was not associated with any specific clinical or prognostic features. The inclusions persisted during antileukemic therapy. Their formation may be related to a dysfunction in the synthesis of surface membrane-bound immunoglobulins. (Author abstract) (29 Refs)

27. STEREOLOGICAL INVESTIGATIONS OF DESMOSOME FREQUENCY DURING ORAL CARCINOGENESIS (MEETING ABSTRACT)

White FH, Gohari K
Department of Oral Pathology, University of Sheffield, Sheffield, England
Br J Cancer; 44(2):302 1981

Desmosomes are membrane specializations which are responsible for intercellular attachment. There are may subjective reports describing alterations in desmosomes in malignant lesions. The development of stereological methods now enables morphology to be placed on a quantitative basis. Thus the aim of this investigation was to quantify desmosome frequency using stereological method during sequential DMBA (dimethylbenzanthracene) carcinogenesis in hamster cheek pouch. After DMBA treatment, pouch samples were removed and epithelial lesions were classified into hyperplasia, dysplasia and carcinoma groups. Untreated pouch epithelium served as a control group. Representative samples from 5 animals in each group were used to obtain electron micrographs of defined basal, spinous and granular cells. Using a test lattice comprising parallel lines, intersections of the lattice lines with desmosomes and plasma membrane were counted. Using these data the number of desmosomes per unit area of plasma membrane (Ns) was estimated from the formula Ns is equal to the absolute value of A x the mean of delta where N is the number of chromosomes, A the surface area on which they are present and the mean of delta is the mean desmosome diameter. Ns values were obtained for basal, spinous and granular layers of each group. The results indicate that during chemical carcinogenesis the parameter Ns decreases by more than 50% in basal and spinous cells. This decrease in desmosomal frequency may be a requirement for carcinoma cell invasion and metastasis. (no Refs)

28. ALTERATIONS AT THE EPITHELIAL-STROMAL INTERFACE IN CARCINOMA OF THE RAT BLADDER (MEETING ABSTRACT)

Memoli VA, Pauli BU

Department of Pathology, Rush, Presbyterian-St. Luke's Medical Center, Chicago, IL

71st Annual Meeting of the International Academy of Pathology (United States-Canadian Division), March 1-5, 1982, Boston, Massachusetts. International Academy of Pathology 95 pp., 1982.

Substantial alterations occur at the epithelial-stromal interface during the course of progression of a tumor from a noninvasive to an invasive state. These alterations at the epithelial-stromal junction in carcinogen (FANFT)-induced noninvasive carcinomas of rat urinary bladder were compared with control normals, using quantitative electron microscopy. Our results indicate an increased number of epithelial interdigitations within submucosal connective tissue in noninvasive and invasive carcinomas relative to controls. Perimeter length of the basal plasma membrane per unit length of submucosal tissue circumference (STC) shows a 56 per cent increase in invasive carcinoma, relative to controls. Although the perimeter of the basement membrane per STC is increased in early noninvasive carcinoma, it is decreased in invasive carcinoma, to 16 per cent of controls. These findings represent not only a marked increase in the contact area between tumor and submucosa, but an increase in the direct contact area between basal tumor cells and connective tissue stroma. This increase in direct contact area may contribute to the efficacy of matrix degradation by membrane-associated proteinases, which have been shown to be increased in invasive rat bladder tumors. The loss of basement membrane in invasive carcinomas is paralleled by a decrease in the number of hemidesmosomes per unit length of basal plasma membrane. Since hemidesmosomes anchor epithelial cells to their basement membrane, it is reasonable to suggest that focal reductions in hemidesmosome numbers, accompanied by decreased numbers of desmosomes, would reduce adhesion between tumor cells and the underlying connective tissue. (no Refs)

MEMBRANE RECEPTOR/ BINDING SITES

29. INHIBITION OF PHORBOL ESTER-RECEPTOR BINDING BY A FACTOR FROM HUMAN SERUM.

Horowitz AD, Greenebaum E, Nicolaides M, Woodward K, Weinstein IB

Div. Environmental Sciences and Cancer Center, Inst. Cancer Res., Columbia Univ., New York, NY, 10032

Mol Cell Biol; 2(5):545-553 1982

The mechanisms by which a factor isolated from human serum (SF) inhibits phorbol-12,13-dibutyrate (PDBu) binding in intact monolayer cultures of the rat embryo cell line CREF N and in a subcellular system containing membranes of these cells, were studied. In addition the release induction of arachidonic acid and choline from prelabeled cellular phospholipids by PDBu was compared to that of the SF. The SF may perform a protective role in host susceptibility to carcinogenesis. (26 Refs)

30. PHORBOL DIESTER RECEPTORS ON HL-60 HUMAN PROMYELOCYTIC LEUKEMIA CELLS (MEETING ABSTRACT)

Lane RW, Coles RB, Carchman RA, Borzelleca JF

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Toxicologist; 2(1):105 1982

Experimentally-induced carcinogenesis involves two separate steps, initiation and promotion. Promotion can be caused by a number of chemicals, the classic compounds being the phorbol diesters. It has recently been demonstrated that there are specific binding sites for the phorbol diesters on the plasma membranes of many different types of cells. Such specific binding sites have also been characterized in our laboratory using HL-60 cells, a continuous cell line of human promyelocytes, with tritiated phorbol dibutyrate (3H-PDBu). Binding was saturable and reversible. The KD was determined to be approximately 50nM with 7.5 x 10(5) sites/cell. Competition for binding sites by other phorbol diesters was directly related to the ability of the compound to promote tumor formation; compounds not related to the phorbol diesters did not compete. The effect of PDBu on cellular function was also examined, using the release of the enzyme N-acetylglucosaminidase (NAGA) as a marker. The release of NAGA increased in both time and concentration. The curves were similar to those obtained for 3H-PDBu binding, exhibiting similar values for the EC50 of NAGA release and KD for binding. Nonpromoting phorbol diesters did not cause NAGA to be released. (no Refs)

31. DECREASED RESPONSE TO PHORBOL ESTERS INVOLVES A TWO-STEP PROCESS INCLUDING RECEPTOR MODULATION (MEETING ABSTRACT)

Jaken S, Phillips M, Tashjian AH

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Toxicologist; 2(1):69 1982

Tumor-promoting phorbol esters (PE) act on cells via interaction with specific membrane receptors. Escape from PE-mediated actions, possibly including tumor promotion, have been documented. We have studied the mechanisms by which cells escape from PE effects using as a model system a clonal strain of mouse pituitary cells (D16) which synthesize and secrete ACTH. PE enhance ACTH release from D16 cells 2-to 5-fold above unstimulated control levels. ED50s for this response, approx 16 nM for phorbol dibutyrate (PDBu), approx 2 nM for phorbol myristate acetate (PMA) agree closely with the apparent Kds of these ligands for the PE receptor as measured by competition for binding with [3H]PDBu. Pretreatment with PE for 2 hr resulted in a decrease in ACTH release with PE rechallenge to 23% of that from cells not pretreated (desensitization). The 2-hr pretreatment did not change the affinity of number of PE receptors nor did it decrease the responsiveness of the cells to other ACTH secretagogues (cAMP, isoproterenol). However, the continued presence of PE caused a gradual decrease in [3H]PDBu binding which was first detected at 5 hr and was maximal by 24 hr. Scatchard analysis indicated a decrease in the number of [3H]PDBu binding sites (down modulation). In these down-modulated cells, the magnitude of PE stimulation of ACTH release correlated well with the number of remaining PE-receptors. (no Refs)

32. MACROMOLECULAR INTERACTIONS OF [14C-RING]MELPHALAN IN BLOOD.

Ahmed AE, Hsu TF, El-Azhary RA, Moawad H, Costanzi J
Dept. Pathology, Pharmacology and Toxicology, Medicine, Univ. Texas Medical Branch, Galveston, TX, 77550
Biochem Pharmacol; 31(8):1615-1619 1982

The pharmacokinetics and macromolecular interactions of [14C-ring]melphalan (L-PAM) in blood were studied in rats following a single oral dose (20 mg/kg, 0.1 mCi/kg). Radioactivity levels were monitored in blood over a period of 72 hr. The highest levels of radioactivity were observed at 2 hr. The decline of radioactivity from the blood was biphasic with T1/2alpha = 7 hr and T1/2beta = 75 hr. The radioactive species in plasma corresponded to unchanged L-PAM and its two known hydrolytic products 4,2-hydroxyethyl 2-chloroethylamino-L-phenylalanine (L-MOH) and 4[bis(2-hydroxyethyl)amino]-L-phenylalanine (L-DOH). In addition, four other major, previously unknown, metabolites of L-PAM were detected in plasma. At 72 hr, most of the radioactivity was bound to macromolecular components, 26% to plasma macromolecules and 62% in red blood cells. Covalent binding to blood cells was mainly to membrane proteins. Binding to hemoglobin and other soluble components of the red cells was also observed, with a 5,000-fold greater affinity for membranes. These studies suggest extensive interaction of melphalan, or its metabolites, with membrane and soluble proteins of red blood cells. (Author abstract) (24 Refs)

33. HEPARIN-BINDING PROPERTIES OF THE EHRLICH ASCITES TUMOR CELL MEMBRANES (MEETING ABSTRACT)

Kremmer T, Rosdy B, Laszlovszky I, Holczinger L
Department of Biochemistry, National Oncological Institute, Budapest, Hungary
VI Meeting of the European Association for Cancer Research, October 12-15, 1981, Budapest, Hungary. The European Association for Cancer Research 275 pp., 1981.

An improved spectrofluorimetric method has been worked out for measuring the in vitro heparin-binding properties of cell membranes using 1-anilino-naphtalene-8-sulfonic acid (ANS) as a fluorescence probe. Ehrlich ascites tumor cell membranes obtained from early (6-7 days) and late (14-16 days) phases of cell proliferation have shown characteristic differences in their heparin-binding properties. Depending on the heparin concentrations applied the fluorescence response of cytoplasmic membranes was more pronounced in the cells of late growth phase. In order to clarify the molecular basis of the cell surface alterations indicated by the heparin titration curves biochemical methods regarding the membrane lipids and (glyco)proteins were employed. (no Refs)

34. THE USE OF AVIDIN-BIOTIN-PEROXIDASE COMPLEX (ABC) IMMUNOPEROXIDASE TECHNIQUE FOR IDENTIFICATION OF CELL SURFACE A, B, OR H BLOOD GROUP ANTIGENS IN BLADDER CARCINOMA: A COMPARISON BETWEEN ABC AND SPECIFIC RED CELL ADHERENCE (SRCA) TECHNIQUES (MEETING ABSTRACT)

Newman AJ, Carlton CE, Murphy WM
No affiliation given
77th Annual Meeting of the American Urological Association, Inc., May 16-20, 1982, Kansas City, Missouri. American Urological Association 254 pp., 1982.

The major blood antigens A, B, or O (H) are present on normal urothelium. The presence or absence of these antigens on the cell surface of bladder tumors, as measured by the specific red cell adherence (SRCA) technique, has been shown to have prognostic value. In a series of 322 patients with superficial bladder tumors, we found that 71 of the 99 patients with a negative SRCA reaction subsequently developed an invasive lesion. While encouraging, this still left a 30 percent false negative rate. In an effort to increase the sensitivity of the assay, we have applied the avidin-biotin-peroxidase complex (ABC) immunoperoxidase technique of Hsu and coworkers to a series of 100 randomly selected formalin-fixed bladder tumor specimens from the prior SRCA study. One of us (WMM) did all of the ABC histochemical staining with no knowledge of the subsequent clinical course which consisted of either no recurrence in 5 years (25 cases), multiple superficial recurrences (25 cases), recurrence with invasion (25 cases); 25 cases represented transitional cell carcinoma with muscle invasion. Based upon recent data by Allen and coworkers showing that the purified Ulex europaeus lectin agglutinates erythrocytes of all three blood groups with little distinction between types O (H) and B, we have utilized biotinylated purified Ulex as the primary antisera in the ABC technique for all blood types. All of the invasive tumors showed delection of blood group antigen as previously found with the SRCA technique. The false negative rate using the ABC technique was only 10 percent (3 of 28). (no Refs)

35. APPLICATION OF LECTINS.

Kuroume T, Oshima Y, Nagashima K, Hiraishi Y, Kuribayashi T
Dept. Pediatrics, Gunma Univ. Sch. Medicine, Gunma Prefecture, Japan
Nippon Rinsho; 39(8):2790-2798 1981

Lectin is used widely for the assay of cell surface markers and for the classification of leukemia cells. Attempts are being made to use the unique binding property of lectin for the determination of the source of cells and for arriving to a very specific differentiation. Using Concanavalin A, the cap formation in malignant lymphoma cells is found to be lower that that in normal lymphocytic cells. Relationship between cap formation in leukemic cells and membrane fluidity is not yet known. The lectin receptors on cells transformed by tumor virus are exposed, whereas those on normal cells are carried, since binding of lectins increases when normal cells are treated with proteases. So far no differences were seen in the lectin receptor of normal cells and in that of malignant cells. Usefulness of lectin, should not be limited only to detect the presence or absence of binding by malignant cells, but rather, should be to recognize the membrane status of the cells binding the lectin, and to observe the changes in cellular metabolism induced by the lectin binding. Further, based on the understanding of membrane properties, efforts must be made to develop test and therapeutic methods. (32 Refs) (In JPN)

36. EPSTEIN-BARR VIRUS-LYMPHOID CELL INTERACTIONS. III. EFFECT OF CONCANAVALIN A AND SACCHARIDES ON EPSTEIN-BARR VIRUS PENETRATION.

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J Virol; 42(2):402-410 1982

To study some aspects of Epstein-Barr virus (EBV) penetration into target cells, the effect of concanavalin A (ConA) and various saccharides on virus infectivity and cell susceptibility to EBV infection was examined. ConA treatment of the target cells, EBV, or EBV-cell complexes was found to inhibit virus

antigen expression. Several control experiments with alpha-D-methyl-mannoside elution of ConA, removal of nonfused EBV particles from the cell surface by trypsin treatment, and addition of ConA at different times postinfection were performed to define the site of ConA action on EBV infection. ConA appeared to have a dual action: (i) it inhibited EBV binding to virus receptors, and (ii) it blocked the penetration of receptor-bound virus into target cells at a trypsin-sensitive stage, thus indicating that ConA prevented the fusion of viral envelope with the target cell membrane. A high sucrose concentration (0.25 M), known to inhibit cell membrane movements, was also found to block EBV penetration at a trypsin-sensitive stage, thus suggesting the implication of cell membrane movements and underlying activities (or both) in viral envelope fusion. Lower concentrations of various monosaccharides (0.12 M) did not influence EBV infection. Under conditions of ConA treatment that did not influence EBV infectivity and target cells susceptibility, ConA was able to mediate virus binding to EBV receptor-negative cell lines, but no virus antigens were expressed in these cells. These observations reinforced the idea that the mere attachment of EBV to lymphoid cells is not sufficient to lead to infection. In light of the present and previously published data, we postulate the existence of a specific cellular mechanism that allows the penetration of EBV into the target (B) lymphocyte. (Author abstract) (37 Refs)

37. FLUORESCEINATED FUCOSE BINDING LECTINS AS MYELOMONOCYTIC CELL SURFACE MARKERS USEFUL IN CHARACTERIZATION OF HUMAN LEUKEMIA (MEETING ABSTRACT)

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Proc Am Soc Clin Oncol; 1:C-15 1982

Two fluoresceinated lectins have been recently studied as markers of normal and malignant myeloid differentiation, using flow cytometry. In previous studies by Van Epps et al, fucose binding lectin from Lotus tetragonolobus (FBL-L) was noted to bind strongly to normal neutrophils, moderately to monocytes, and not to lymphoid cells. Lectin from Ulex europeaus I (FBL-U) bound to normal monocytes and a lymphoid subpopulation but not normal granulocytic cells or precursors. Binding of either was reversible by L-fucose. In this study, binding to human malignant cells was compared to that of monoclonal antibodies including OKM1 and alpha Mono. Chronic lymphocytic leukemia cells were negative for both lectins. Chronic myeloid leukemia (CML) cells were FBL-L+, FBL-U-, OKM1+, and alpha Mono-. Four adult patients with morphologically undifferentiated or lymphoblastoid acute leukemias including one with CML blast crisis were studied. All were OKM1-, alpha Mono- however one was FBL-L+, FBL-U- and one FBL-L+, FBL-U+. Among cell lines, K562 (an undifferentiated nonlymphoid line) was weakly FBL-L+, HL-60 (a promyelocytic line) and U-937 (a monoblastic line) were FBL-L+. Incubation with differentiation inducing agents such as dimethylsulfoxide and conditioned medium resulted in increases of FBL-L binding of HL-60 and U-937 but no FBL-U binding was noted under any condition. Patterns of binding of other markers were complex but divergent compared to the FBL's. (no Refs)

38. KINETICS OF INTERNALIZATION AND RECYCLING OF THE ASIALOGLYCOPROTEIN RECEPTOR IN A HEPATOMA CELL LINE.

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J Biol Chem; 257(8):4230-4237 1982

The presence of a large number of functional asialoglycoprotein receptors in the human hepatoma cell line Hep G2 has allowed us to determine the parameters which define ligand binding to the surface receptor, ligand receptor internalization, and receptor recycling. We have developed a simple kinetic model which encompasses all of these processes. These cells contain 225,000 functional asialoglycoprotein receptors, and at 37 C uptake at a half-saturating concentration (2 ug/ml) of 125I-asialoorosomucoid (ASOR) is linear for several hr at a rate of 15,000 molecules/cell/min. Neither the rate of 125I-ASOR binding at 4 C nor the rate of uptake at 37 C was altered under conditions in which protein synthesis was completely inhibited. Thus each receptor recycles a ligand, on the av, every 16 min. The rate of binding of 125I-ASOR to cells at 37 C, independent of internalization, was measured in the presence of 10-12 mM azide. The rate of internalization at 37 C was determined from cells, prebound with ligand at 4 C, by following EDTA-resistant cell-associated 125I-ASOR. The distribution of functional receptors within the cell was assessed from ligand binding at 4 C and ligand uptake at 37 C in cells pretreated with trypsin to remove all surface receptors. In growing Hep G2 cells 87% of the receptors are on the cell surface, and over 88% of these are unoccupied with ligand. During steady state internalization of 125I-ASOR, the number of surface ASOR receptors decreases 17%. At 2 ug/ml of 125I-ASOR, an unoccupied surface receptor requires a mean time of 8.7 min to bind a ligand, and a mean time of 2.2-2.3 min is required to internalize a surface receptor-ligand complex. An additional 4.2 min is required for ligand to dissociate from an internalized receptor and for the receptor to return to the cell surface. We have been able to determine or calculate most of these parameters of receptor recycling by two or more independent sets of measurements. (Author abstract) (24 Refs)

39. A NEW KUPFFER CELL RECEPTOR MEDIATING PLASMA CLEARANCE OF CARCINOEMBRYONIC ANTIGEN BY THE RAT.

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Biochem J; 204(2):377-381 1982

Native human carcinoembryonic antigen is rapidly removed from the circulation by the rat liver Kupffer cells after intravenous injection. The molecule is subsequently transferred to the hepatocyte in an immunologically identifiable form. Carcinoembryonic antigen has a circulatory half-life of 3.7 (+-0.8)min, and cellular entry is by receptor-mediated endocytosis. Non-specific fluid pinocytosis and phagocytosis can be excluded as possible mechanisms by the kinetics of clearance and failure of colloidal carbon to inhibit uptake. Substances with known affinity for the hepatic receptors for mannose, N-acetylglucosamine, fucose and galactose all fail to inhibit carcinoembryonic antigen clearance. After two cycles of the Smith degradation, carcinoembryonic antigen is still able to inhibit clearance of the native molecule. Receptor specificity is apparently not dependent on those non-reducing terminal sugars of the native molecule. Performic acid-oxidized carcinoembryonic antigen also inhibits clearance of carcinoembryonic antigen in

vivo. Receptor binding is not dependent on tertiary protein conformation. Non-specific cross-reacting antigen, a glycoprotein structurally similar to carcinoembryonic antigen, is cleared by the same mechanism. (Author abstract) (30 Refs)

40. RECEPTOR INTERACTIONS OF NERVE GROWTH FACTOR.

Riopelle RJ, Sutter AK, Harris-Warrick RM, Klearman M, Shooter EM, Hall, Weston
Dept. Medicine (Neurology), Queen's Univ., Kingston, K7L 3J7 Ontario, Canada
Adv Neurol; 29:133-148 1981

The chemical characteristics of the components of receptors for nerve growth factor (NGF) found on dorsal root ganglia and sympathetic cells, and binding heterogeneity, are discussed. Results for kinetics of binding of beta-NGF to day 8 chick dorsal root ganglion membrane preparations are discussed in relation to observed nonlinearity of Scatchard plots. The number and roles of binding sites and the possibility that NGF is processed following binding are considered. (30 Refs)

41. CHEMOTAXIS OF GRANULAR LEUKOCYTE: ITS BIOCHEMICAL AND MORPHOLOGICAL MECHANISM.

Hirashima M, Hirotu Y, Hayashi H
First Dept. Pathology, Kumamoto Univ. Sch. Medicine, Kumamoto, Japan
Nippon Rinsho; 39(8):2701-2708 1981

Two neutrophil chemotactic factors (NCF) are known: the primary factor leucoegresin (mol wt 140,000) and the secondary factor (mol wt 14,000) a derivative of C5. Both are thermoresistant. Artificial peptides, such as f-Met-len-phe, also serve as NCF. Others were also found, but were thought to be less important. Eosinophilic chemotactic factor (ECF) consists mainly of thermolabile early factor (mol wt 500) and thermolabile delayed factor (mol wt 70,000). The early factor may be the ECF-A (ECF of anaphylaxis) or a similar molecule. The delayed factor can be further separated into two different antigens: a and b. Various other factors were also found. All basophilic chemotactic factors studied so far are the results of the in vitro experiments, and no studies have yet been performed with a natural mediator. The two types of leukocyte movement are the random movement occurring in a medium without concentration gradient of the chemotactic factors (CF), and the directional chemotactic movement that initiates the gradient of CF concentration. The latter movement is initiated when CF binds CF receptors on the membrane to form lamellipodium as the forward section. The binding of CF to the CF receptor triggers several biochemical reactions, acting on microfilaments and microtubules, which are moved around within the cell due to gradient changes of Ca++ and Na+. (45 Refs) (In JPN)

42. INTERFERON-INDUCED CHANGES IN THE EXPRESSION AND TOPOGRAPHY OF H-2 ANTIGENS AND RECEPTORS FOR SRBC IN TUMOUR CELLS.

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Folia Biol (Praha); 28(1):28-42 1982

The expression of the H-2 antigens and its changes after interferon (IFN) treatment were studied in methylcholanthrene (MCA) and mouse sarcoma virus(MSV)-transformed clones of C3H mouse embryonic fibroblasts. Topography of H-2 antigenic determinants on the cell membrane and the availability of receptors for sheep red blood cells (SRBCs) were also studied. Both MCA- and MSV-treated cells absorbed anti-H-2.23 (H-2Kk) antibody more effectively than did the control C3H cells; the MCA-treated cells were more effective than the MSV-treated clone. After IFN treatment, one MCV-treated clone showed increased absorbing capacity for H-2DK antibody, and that clone plus another showed increased absorbing capacity for H-2Kk antibody. The effect of IFN on the topography of H-2 determinants as measured by the blocking index was variable, depending upon the MCA-treated clone used and the genetic determinants of H-2 antigenic activity responding to the antisera used. Rosette-forming capacity with SRBCs differed between the two MCA-treated clones; one responded to IFN treatment by greater rosette formation; the other did not. Enhancement of H-2 antigen expression after IFN treatment apparently was the result of change in the spatial relationship of H-2 determinants on the cell surface rather than an actual increase in the number of H-2 antigenic receptors. (37 Refs)

43. STUDY OF IGE AND IGG FC SURFACE RECEPTORS ON MOUSE MASTOCYTOMA CELLS (MEETING ABSTRACT)

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Fed Proc; 41(3):825 1982

The mastocytoma cell line MMC-1 derived from a tumor in CXBI mice releases histamine with both IgE and IgG mediated reactions. The nature of the Fc(epsilon) and Fc(gamma) receptors on these cells was investigated. To study the Fc(gamma) receptor we used a rabbit antiserum of the IgG2b receptor on the mouse macrophage P388D cell line. The Fc(epsilon) receptors were assayed using either rat IgE or rabbit antiserum to the Fc(epsilon) receptor of rat basophil leukemia cells. Fluorescent studies demonstrated simultaneous binding of rat IgE and antiserum to Fc(gamma) receptors, thus a single cell expressed both Fc(epsilon) and Fc(gamma) cell surface receptors. Cells were surface labeled with 125I, the membranes solubilized, and the Fc-IgG and Fc-IgE receptors immunoprecipitated and analyzed on sodium dodecyl sulfate polyacrylamide gels. IgE and anti-IgE or rabbit anti-Fc (epsilon) precipitated a broad band of protein with an apparent molecular weight of approx 60K. Anti-Fc-IgG antiserum precipitated a similar protein peak with a slightly higher apparent molecular weight (68-74K). By sequential immunoprecipitation, removal of the membrane component(s) reacting with rat IgE or antiserum to the Fc(epsilon) receptor left behind a component(s) precipitating with anti-Fc(gamma). Therefore, the FC(epsilon) and Fc(gamma) receptors are antigenically distinquishable and appear to be separate cell membrane components. (no Refs)

44. IGG FC RECEPTORS IN SURFACE MEMBRANE OF HUMAN GLIAL TUMORS (MEETING ABSTRACT)

Tsai CC, Blank WF, Roodman ST
St. Louis University, St. Louis, MO
Abstracts of the 58th Annual Meeting, June 11-13, 1982, Philadelphia, Pennsylvania. American Association of Neuropathologists 386 pp., 1981.

In order to further understand the membrane characteristics of human glial tumors, we have studied surface membrane receptors in frozen sections and tissue culture of human brain tumors using sheep erythrocytes (E), IgG-EA and IgM-EAC rosette techniques. Strong IgG EA rosetting (IgG Fc receptors) were found in the neoplastic cells of 14 patients with

glioblastoma multiform and 8 with astrocytoma. Other brain tumors including medulloblastoma, ependymoma, meningioma, pinealoma and metastatic carcinomas failed to show the similar findings. Control brain tissues from the same patients, 'normal' brain of variable age group and reactive glial foci in cerebral infarcts, hemorrhage, viral infection and metastatic lesions did not express the IgG Fc receptor sites in the glial cells whereas foamy macrophages showed strong EA rosettings The IgG Fc receptors in the neoplastic cells is not species specific since they bind to human, rabbit and goat IgGs. The receptors do not form rosettes with IgM-EA or EAC. They can be demonstrated by direct and indirect immunofluorescence using aggregated human IgG and IgG immune complexes but not with IgG-F(ab)2. The receptors persist in tissue culture for up to three months. Pretreatment with aggregated IgG and trypsin markedly inhibit or abolish the IgG EA rosette formation. These receptors are heat labile and destroyed by fixation in alcohol, formalin and glutaldehyde. (no Refs)

TRANSPORT STUDIES

45. INTERRELATION BETWEEN MEMBRANE TRANSPORT AND THE CONTENTS OF ALKALI METAL CATIONS IN HELA CELLS.

Ikehara T, Saki T, Miyamoto H, Kenlike K
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Jpn J Physiol; 32(1):13-24 1982

The relation of membrane transport of alkali cations to their external concentration or to their cellular contents was studied in HeLa cells. Rubidium ion uptake into cells that were rewarmed after chilling in an ice bath was 2x higher than uptake into control cells and was suppressed by ouabain. Cell Na+ increased almost to the level of cell K+ after chilling, but decreased rapidly after the start of rewarming. Cell K+ decreased after chilling and increased rapidly after rewarming. Chilling to stimulate Rb+ pumping in HeLa cells may be due to the stimulation of the activity of the cation pump, and/or an increase in the number of pump sites. (32 Refs)

46. ANALYSIS OF THE TRANSPORT SYSTEM FOR INORGANIC ANIONS IN NORMAL AND TRANSFORMED HEPATOCYTES.

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J Biol Chem; 257(8):4381-4385 1982

The transport system for inorganic anions has been investigated in hepatocytes and hepatoma tissue culture cells. Sulfate transport in hepatocytes is temperature sensitive and occurs against an electrochemical gradient. Uptake was shown to occur by a sodium-dependent and a sodium-independent route with Km values of 2.3 and 33 mM and Vmax values of 2.1 and 10nmol/mg of protein/min, respectively. An analysis of the sodium dependency indicates a Hill coefficient of 1.05 suggesting an equimolar stoichiometry for sodium and sulfate transport. The transport of sulfate was decreased by metabolic and sodium transport inhibitors. Bicarbonate was shown to affect the transport of sulfate, where uptake was accelerated by intracellular bicarbonate and competitively inhibited by extracellular bicarbonate. In addition, sulfate efflux was stimulated by extracellular bicarbonate. These results suggest that bicarbonate is a substrate for the sulfate transport system and can accelerate uptake and efflux by an anion exchange mechanism. Inhibition of bicarbonate uptake by extracellular sulfate and by the anion transport inhibitor 4,4'-diisothiocyano-2,2'-stilbene disulfonate demonstrates that bicarbonate does not enter the cell exclusively by CO2 diffusion but can be transported in part as an anionic species. These results are consistent with its role in the sulfate-bicarbonate exchange system. This inorganic anion transport system was shown to be inhibited by approx 80% in hepatoma tissue culture cells where altered sodium dependency, Km, and Vmax values reflect possible alterations in the structure and/or membrane content of the carrier. (Author abstract) (30 Refs)

47. EFFECT OF PH ON PHOSPHATE TRANSPORT IN EHRLICH ASCITES TUMOR CELLS (MEETING ABSTRACT)

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Department of Physiology, University of Texas Health Science Center, San Antonio, TX, 78284
Fed Proc; 41(3):671 1982

Inorganic phosphate (Pi) transport is dependent on extracellular Na+ and is saturable (Ks equals 35 mM Na+; 37 C, pH 7.4). About 15% of the total flux is Na+-independent. Saturation kinetics are also found with respect to extracellular Pi (Ks equals 0.11 mM, Jmax equals 1.73 mmol/kg/min) when extracellular Na+ is 150 mM. Since at pH 7.4 Pi exists as H2PO(4)- (16% of total) and HPO(4)-- (84%), the effect of pH on Pi transport was studied to determine which form is transported. Steady state Pi exchange flux at 37 C was measured as a function of extracellular Pi at pH 5.6 and 7.9. At pH 5.6 Ks equals 0.057 mM and Jmax equals 0.38 mmol/kg/min while at pH 7.9, Ks equals 0.57 mM and Jmax equals 1.04 mmol/kg/min. A small linear component may be present in both cases. Pi transport as a function of pH was then measured while holding either [H2PO(4)-] or [HPO(4)--] constant at 0.5 mM by varying total extracellular [Pi]. With [H2PO(4)-] contant, Pi flux vs [HPO(4)--] exhibited saturation-type kinetics with Ks equals 0.15 mM. This conflicts with the value found at pH 7.9 where HPO(4)-- is 94% of the total Pi. A plot of Pi flux vs [H2PO(4)-] at constant [HPO(4)--] was biphasic with an early rising phase followed by a decline of over 50%. For the rising phase, apparent Ks equals 0.05 mM, which is similar to the results at pH 5.6 where H2PO(4)- is 93% of total Pi. It is suggested that the H2PO(4)- specie is transported by the Na+- dependent system and that H+ can inhibit the transport process. (no Refs)

48. CALCIUM UPTAKE IN MASTOCYTOMA P-815 CELLS.

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Biochim Biophys Acta; 687(2):179-188 1982

Kinetic data of 45Ca++ exchange in exponentially growing mastocytoma P-815 cells at 37 C indicated the presence of at least two calcium compartments, fast and slowly exchangeable. The fast phase of calcium exchange at the cell surface and a rate constant of 36.1 min-1 and a compartment size of 8.27 nmol/10(6) cells, while the slow phase, the intracellular calcium exchange, showed a rate constant of 2.4 min-1 and a compartment size of 0.53 mnol/10(6) cells. The fast phase comprised two calcium binding sites of high and low affinities with dissociation constants of 0.59 and 4.88 mM, respectively, and binding maxima of 5.15 and 34.4 nmol/10(6) cells, respectively. The calcium uptake at both binding sites was inhibited by 80%

on the addition of ruthenium red (0.4 mM) or lanthanum chloride (0.5 mM), but not by tetracaine (5 mM). The high-affinity site had two negative reaction enthalpy changes, while the low-affinity site showed two positive reaction enthalpy changes, suggesting the effect of membrane fluidity on calcium binding. The slow phase of calcium exchange was also inhibited by ruthenium red and lanthanum chloride resulting in a decrease of the Hill's coefficient from 1.70 to 1.28 and 1.13, respectively, but was not affected by tetracaine. Calcium efflux from 45Ca-loaded cells was also inhibited by ruthenium red and lanthanum chloride and was also temperature sensitive. The efflux rate constant was reduced by 80% and 100% at 20 C and 0 C, respectively. As calcium uptake fluctuated during the cell cycle of synchronous mastocytoma P-815 cells with a peak at the mid S phase and the lowest level during the late M phase, the compartment size of fast phase also fluctuated, being highest at the S phase and lowest at the M phase. In contrast, the compartment size of the slow phase was highest at the G1 phase and lowest at the M phase. In parallel with these changes, the binding max of the high-affinity site was highest at the S phase and lowest at the M phase, while that of the low-affinity site fluctuated little throughout the cell cycle. (42 Refs)

49. PLASMA MEMBRANE CALCIUM PERMEABILITY IN TUMOUR CELLS (MEETING ABSTRACT)

Cittadini A, Dani AM, Wolf F, Russo MA
Institute of General Pathology, Catholic University, Rome, Italy
VI Meeting of the European Association for Cancer Research, October 12-15, 1981, Budapest, Hungary. The European Association for Cancer Research 275 pp., 1981.

Plasma membrane Ca++ permeability has been investigated in intact ascites thymoma cells compared to normal thymus cells. In tumor cells complete energy depletion is necessary to observe Ca++ entry and equilibration with external Ca++, as revealed by time course cell Ca++ accumulation experiments performed under different metabolic conditions. Ca++ ionophore A23187 is without effect both in control and energy depleted cells. In normal thymocytes Ca++ equilibration across plasma membrane occurs only in the presence of A23187 irrespective of cell energy conditions. The study of the 45Ca++ flux confirms these findings. The data suggest that in cancer cells plasma membrane permeability to external Ca++ is not a limiting step in the regulation of intracellular Ca++ content and concentration; the only mechanism that prevents uncontrolled intracellular Ca++ rise is the outwardly directed energy-supported pump. Ultrastructural aspects of the observed phenomena will be presented. The data altogether will be discussed in relation to the role that calcium may play in the modulation of cancer cell biological and biochemical features. (no Refs)

50. CHANGES IN AMINO ACID AND SUGAR TRANSPORT ACTIVITY ASSOCIATED WITH MALIGNANT TRANSFORMATION.

Nishino H, Inui K
Dept. Biochemistry, Kyoto Prefectural Medical Univ., Kyoto, Japan
Tanpakushitsu Kakusan Koso; 26(7):881-895 1981

Changes in the membrane transport of amino acids and sugars by transforming cells in many systems were reviewed. Increase in membrane transport is usually due to the increase of transport carriers as the cell membrane changes. To study such mechanism, the method of using membrane vesicles made from purified membrane has been developed and widely applied to the amino acid transport system. This membrane vesicle system eliminates the complication of metabolism associated with the whole cells. Such system is also increasingly applied to to the study of sugar transport as well. Other useful techniques are also developed, such as purification of transport carriers and membrane, and creation of an in vitro system by artificially combining the two components. Some salient results were presented and discussed. (75 Refs) (In JPN)

51. CONTRASTS IN TRANSPORT SYSTEMS FOR ANIONIC AMINO ACIDS IN HEPATOCYTES AND A HEPATOMA CELL LINE HTC.

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J Biol Chem; 257(10):5663-5670 1982

The uptake of anionic amino acids by the rat hepatoma cell line, HTC, occurs by two saturable routes, one Na+-dependent and one Na+-independent. Which of the two mediations will serve for transport of a given anionic amino acid depends on the chain length of that anionic substrate. The Na+-independent system, shared by glutamate, cysteine, and anionic amino acids intermediate in length between the two, is stereoselective, pH-independent, and not inhibited by neutral amino acids. The pH-independent inhibition of this system by LL-beta-mercaptolactate-cysteine disulfide confirms that cystine reacts as an anionic amino acids. Shorter anionic amino acids, including aspartate, are scarcely transported by this route. The accommodation of the cystine molecule calls for its stabilization as an anion even below pH 7. Inhibition triple that of cysteine by the product of its hydrolytic monodeamination confirms this interpretation. A Na+-independent system similar to the one described here has been reported for fetal human lung fibroblasts. In contrast, the Na+-dependent route for anionic amino acid uptake by HTC cells is shared only by the three short anionic amino acids, aspartate, cysteate, and cysteinesulfinate, among the analogs tested. Characteristics of the latter system include an unexplained type of pH dependence and inhibition by threonine and other amino acids known to be substrates for System ASC. Both systems for anionic amino acid uptake in the HTC cell have properties different from those of the two saturable, Na+-dependent routes of anionic amino acid uptake previously described for the cultured rat hepatocyte. (Author abstract) (28 Refs)

52. THE USE OF HG++ TO EVALUATE THE CO-SUBSTRATE/AMINO ACID TRANSPORT STOICHIOMETRY IN EHRLICH ASCITES TUMOR CELLS (MEETING ABSTRACT)

Dawson WD, Robinson SC, Smith TC
Department of Physiology, The University of Texas Health Science Center, San Antonio, TX, 78284
Fed Proc; 41(3):1155 1982

Recent investigations have indicated that cellular rheogenic properties may interfere with the correct estimation of Na+ and amino acid transport stoichiometry. We have reevaluated the stoichiometry of Na+ and alpha-aminoisobutyric acid (alpha-AIB) cotransport in Ehrlich ascites tumor cells depleted of Na+ and ATP by incubation in Na+-free HEPES-buffered medium (pH 7.2) containing 160 mM K+ and 2.5 uM valinomycin. Transfer of the cells to a medium with 10 mM 22Na+, 10 mM 3H-alpha AIB and 150 mM K+ resulted in an enhancement of Na+ flux above basal levels which represents 0.6 of the AIB uptake. HgCl2 (10 uM) added to the medium inhibited AIB flux and AIB-stimulated Na+ flux by 45-50%

but did not change the coupling ratio. HgCl2 (10 uM) does not inhibit the basal Na+ flux (O AIB) nor does it affect cellular Na+ or K+ content. These results are consistent with a model in which the stoichiometry is one co-substrate molecule per molecule of alpha-AIB. We suggest that H+ provides the alternative co-substrate in this low Na+ environment and that in high Na+ medium the Na+: AIB stoichiometry approaches 1:1. Furthermore, since Hg++ depresses both Na+ and AIB uptake (0.6:1) it is unlikely that rheogenic effects of the cotransport have altered the basal Na+ flux. (no Refs)

53. RADIATION EFFECTS ON MEMBRANES. I. CELLULAR PERMEABILITY AND CELL SURVIVAL.

Khare S, Jayakumar A, Trivedi A, Kesavan PC, Prasad R
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Radiat Res; 90(2):233-243 1982

The effect of gamma radiation (GR) on the cellular permeability of the unicellular eukaryote, Candida albicans, was studied. When exponentially growing cultures of C albicans, grown on minimal medium, were exposed to different doses of GR (5, 10, 20, and 60 kilorad), the LD50 was observed to be about 8 kilorad. The LD50's for cells grown in different alkanes (tri-, tetra-, penta-, hexa- or octadecane) was significantly greater (13-18 kilorads). These results suggest that the change in lipid composition of alkane-grown cells exerts some protection against GR-induced damage. GR significantly reduced uptake of proline, glycine, lysine, and glutamic acid in normal glucose-grown cells. Permeases apparently varied in their radiosensitivities, since different doses of radiation were required to cause 50% reduction in the uptake of different amino acids. There was a significant increase in the Km values for proline and glycine following GR. Levels of accumulation of proline and lysine were reduced 45-50% in alkane-grown cells. Compared to glucose-grown cells, levels of accumulation of proline and glutamic acid was not severely reduced by GR. Uptake of lysine exhibited resistance at higher but not at lower doses of radiation. These results suggest that changes in lipid composition affect radiosensitivity of permeases responsible for amino acid uptake. Cellular oxidation was found to be significantly lower in alkane grown cells exposed to radiation than to comparably exposed glucose-grown cells. (20 Refs)

54. USE OF ESTRADIOL-CONTAINING LIPOSOMES FOR THE SELECTIVE TRANSPORT OF SUBSTANCES INTO THE CELLS OF ESTROGEN-DEPENDENT TISSUES AND TUMORS.

Morosova TM, Budker VG, Gutkina NI, Moiseeva GI, Saiganik RI
Inst. Cytology and Genetics, Novosibirsk, USSR
Eksp Onkol; 4(2):47-50 1982

An attempt was made to incorporate estradiol (E) into liposome membrane and thus to enhance the selective transport of E into the estrogen-dependent target tissues. Ovariectomized DD mice bearing mammary gland carcinoma were inoculated iv with free E or with E-containing liposomes. Incorporated E showed more rapid binding to the uterus and liver than the free E. E-containing liposomes showed more intensive incorporation into uterus tissue than E-free liposomes. (15 Refs) (In RUS)

55. EFFECTS OF OUABAIN AND OSMOLARITY ON BUMETANIDE-SENSITIVE POTASSIUM TRANSPORT IN SIMIAN VIRUS-TRANSFORMED 3T3 CELLS.

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Biochim Biophys Acta; 687(2):333-336 1982

Unidirectional potassium influx in simian virus-transformed 3T3 cells was dissected into a ouabain-inhibitable 'pump' component, a bumetanide-sensitive and chloride-dependent 'cotransport' component, and a residual 'leak' flux. The bumetanide-sensitive component was stimulated 2-3-fold by a 60-min preincubation with ouabain. Subsequent washing of the cells and incubation in ouabain-free saline reversed both the inhibition of the Na+ pump and the stimulation of bumetanide-sensitive flux. Bumetanide-sensitive potassium influx was also stimulated by hypertonic cell shrinkage (induced by 0.1 M or 0.2 M sorbitol). This latter observation suggests that the bumetanide-sensitive system may play a role in cellular vol regulation. (Author abstract) (14 Refs)

56. INHIBITION OF URIDINE TRANSPORT THROUGH SARCOMA-180 CELL MEMBRANE BY ANTHRACYCLINE ANTIBIOTICS.

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Biochem Pharmacol; 31(9):1811-1814 1982

Effects of adriamycin (ADR), nogalamycin (NG), and 7-OMEN on active transport of small precursor molecules through sarcoma-180 ascites tumor cell membrane were examined. NG and ADR inhibited uptake of 3H-uridine (3H-U) by sarcoma-180 cells following an initial lag period. When NG, ADR, or 7-OMEN was preincubated with cells prior to addition of uridine, each antibiotic exhibited dose-dependent inhibition of 3H-U uptake; the effect of NG was more pronounced than that of ADR or 7-OMEN. Acid-soluble extracts from sarcoma-180 cells labelled with 3H-U at 20 C in the presence and absence of NG were subjected to chromotography to separate different uridine nucleotides. No difference in the relative distribution of radioactivity was observed among various nucleotides in control and drug-treated cells. Inhibition by NG and ADR appeared to be non-competitive. The antibiotics seemed to act by binding at a site different from the uridine binding site of the carrier protein. Inhibition of 2-deoxy-glucose uptake by sarcoma-180 cells was observed in the presence of NG and ADR. Although DNA intercalation and subsequent inhibition of transcription is apparently the primary mode of inhibition by the anthracycline antibiotics, cellular toxicity of the antibiotics may result partially from alteration of transport properties of the cellular plasma membrane. (29 Refs)

57. THE EFFECTS OF NITROGEN MUSTARD (HN2) ON ACTIVITIES OF THE PLASMA MEMBRANE OF PC6A MOUSE PLASMACYTOMA CELLS.

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Biochem Pharmacol; 31(9):1773-1778 1982

Nitrogen mustard, HN2 (10(-5) M), inhibited the transport of the potassium congener 86rubidium into PC6A mouse plasmacytoma cells by 45% after a 4 hr incubation at 37 C in vitro. HN2 (10(-3) M) had a rapid effect on the profile of 86rubidium transport into PC6A cells when added simultaneously with the

86 rubidium whereas a monofunctional analogue of HN2 ((2-chloroethyl)dimethylamine) had no effect at 10(-3) M. The transport of the amino acid analogues alpha-aminoisobutyric acid and cycloleucine into PC6A cells was inhibited by 19% and 5% respectively after a 4 hr incubation with 10(-5) M HN2. The results suggest that the activity of plasma membrane Na+K+-ATPase may be affected by HN2. This enzyme may play a pivotal role in controlling cell growth and division. Crude cell membrane preparations from PC6A cells had variable Na+K+-ATPase activity which was possibly due to contamination with mitochondrial Mg++-ATPase. Incubation of a crude cell membrane preparation in the presence of 40 nM dicyclohexylcarbodiimide gave constant Na+K+-ATPase activity which was inhibited by 44% on incubation with HN2 (10(-3) M) for 0.5 hr. The monofunctional analogue of HN2 inhibited this preparation by only 7% under the same conditions. It is suggested that inhibition of Na+K+-ATPase by HN2 may be an important facet of its cytotoxic activity. (Author abstract) (40 Refs)

58. IN VITRO STUDIES ON THE ABSORPTION OF WR-2721 BY TUMORS AND NORMAL TISSUES.

Ritter MA, Brown DQ, Glover DJ, Yuhas JM
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Int J Radiat Oncol Biol Phys; 8(3/4):523-526 1982

Single-cell system models were studied for possible use in studies of the absorption of the radioprotectant WR-2721. Single-cell suspensions (SCS) were made from normal liver and kidney tissues of BALB/c mice and Fisher 344 rats by means of mechanical dissociation methods. SCS of tumor tissues were prepared by mechanical and, in some cases, enzymatic dissociation. Human fibroblast cultures were prepared from diploid CC105 cells and from HeLa cells. Cultures were exposed to 1 or 2 uM WR-2721 or sodium thiosulfate for 0.5 hr before and during irradiation with 137Cs gamma-rays. Cultures were then incubated for 2 wk. Radiation survival was measured as colony-forming ability. SCS of normal rat and mouse tissues prepared by mechanical means were suitable for studying absorption of WR-2721. These cultures concentrated the drug against a gradient; absorption was reduced at 4 C, indicating that the cells concentrated WR-2721 by metabolic action. Rat RBC absorbed WR-2721 only passively. Bone marrow, which responded strongly to WR-2721 in vivo, responded poorly in vitro. Poor absorption was due to contamination of cell cultures with RBC and lipid. Use of tumor cell SCS was limited by low cell yields, contamination by non-tumor cells of host origin, and possible disruption of cell membranes. WR-2721 protected monolayer cultures of human fibroblasts only slightly from radiation damage and not at all from DNA strand breaks. The tumor and human fibroblast models studied are evidently not suitable for studies of the absorption of WR-2721. (12 Refs)

59. CIRCUMVENTION OF THE TUMOR MEMBRANE BARRIER TO WR-2721 ABSORPTION BY REDUCTION OF DRUG HYDROPHILICITY.

Yuhas JM, Davis ME, Glover D, Brown DQ, Ritter M
Dept. Radiation Therapy, Univ. Pennsylvania, Philadelphia, PA, 19104
Int J Radiat Oncol Biol Phys; 8(3/4):519-522 1982

The relationships between structural and biophysical characteristics of the radioprotectant aminopropylaminoethylphosphorothioate (WR-2721) and the limited ability of WR-2721 to cross the cell membrane of tumor cells was studied. Multicellular tumor spheroids (MTS) of NB-100 human neuroblastoma tumor cells and rat RBC were used for absorption studies. Cell cultures were exposed in vitro to labeled doses of WR-2721, dephosphorylation products of WR-2721, B-mercaptoethylamine (MEA), cysteine or sodium thiosulfate (STF). All drugs were administered at initial concentrations of 10 uM. Octanol-water partition coefficients (PE) were calculated for all drugs. Addition of polyamines to WR-2721 did not inhibit absorption of the drug by normal cells or stimulate absorption by tumor cells. The PE of WR-2721 was between 0.00065 and 0.00045, indicating that the drug is very hydrophilic. Two dephosphorylation products of WR-2721, designated RSSR and RSH, had PE of 0.004 and 0.002, respectively, indicating that these two compounds were less hydrophilic. A mixture of RSSR and RSH showed 2.6x greater absorption by tumor cells at 37 C and 2x greater absorption at 4 C than did WR-2721. MEA and cysteine, both of which had PE greater than 0.001, were readily absorbed by normal and tumor cells. STF, which is very hydrophilic, was not readily absorbed by normal or tumor cells. Data indicate that hydrophilicity affects the absorption of drugs by cells, and that differences in absorption by normal and tumor cells allows selective absorption of WR-2721 by normal cells. (9 Refs)

60. FURTHER STUDIES OF STEREOSPECIFICITY AT CARBON 6 FOR MEMBRANE TRANSPORT OF TETRAHYDROFOLATES. DIASTEREOISOMERS OF 5-METHYLTETRAHYDROFOLATES AS COMPETITIVE INHIBITORS OF TRANSPORT OF METHOTREXATE IN L1210 CELLS.

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Biochem Pharmacol; 31(8):1527-1530 1982

The unnatural d diastereoisomer at carbon 6 of 5-methyltetrahydrofolate was only slightly less effective than the natural l diastereoisomer as a competitive inhibitor of the carrier-mediated membrane transport of [3H]methotrexate into L1210 murine leukemia cells. The apparent Ki for a mixture containing equal amounts of both natural and unnatural diastereoisomers was not significantly different from that found for the unnatural form. These results show that the reduced folate carrier system in these cells has a strong affinity for the unnatural stereoisomer, a finding in contrast to that obtained with the corresponding diastereoisomer of 5-formyltetrahydrofolate. (Author abstract) (24 Refs)

61. A MODEL RELATING DRUG TRANSPORT, PROLIFERATION-DEPENDENCE OF CYTOTOXICITY AND THE EMERGENCE OF RESISTANCE TO THE CURRENTLY USEFUL ALKYLATING AGENTS (AA) (MEETING ABSTRACT)

Byfield JE, Calabro-Jones PM
University of California, San Diego, CA
Br J Cancer; 44(2):279 1981

Using human T-lymphocyte clonogenesis in vitro we have studied the effect of the induction of proliferation on the sensitivity of T-cells to AA killing. It was found that resting cells are considerably more resistant to some agents (melphalan (MEL), cis-platinum, HN2) than cycling cells. No effect of the cell proliferative state could be found for BCNU (carmustine), Me-CCNU (semustine), Mitomycin C, Procarbazine, ACNU (nimustine) and x-rays. The toxicity of MEL was reduced by competition with normal amino acids, confirming the work of

others. This confirms that MEL is probably taken up by amino-acid transport carriers in normal human cells. Glucose-containing drugs and activated cyclophosphamide (phosphoramide mustard) were ineffective against these mature T cells, though cytotoxic against epithelial cells in culture. The data is consistent with a model in which the clinically useful AA fall into two distinct groups: (1) those drugs that are water-soluble, effectively lipid insoluble and whose entry into cells is (membrane) carrier-dependent; (2) those drugs that are lipid soluble or amphipathic (carrier-independent, CID) and that enter cells by simple diffusion. CID agents all show prolonged marrow depression and generally reduced therapeutic ratios. We believe this relates to a greatly reduced dependence on proliferation, since these must also directly enter normal resting (marrow and gut) stem cells. The model permits a rationalization of several aspects of AA anti-cancer activity including drug design. (no Refs)

62. CORRELATION BETWEEN MEMBRANE PERMEABILITY AND INDUCTION OF ERYTHROID DIFFERENTIATION BY DMSO IN FRIEND VIRUS TRANSFORMED ERYTHROLEUKEMIA CELLS (MEETING ABSTRACT)

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No affiliation given

10th International Symposium for Comparative Research on Leukemia and Related Diseases, August 31-September 4, 1981, Los Angeles, California. International Association for Comparative Research on Leukemia and Related Diseases 187 pp., 1981.

We have measured the uptake of phosphate, leucine, uridine, thymidine and deoxyglucose by FEL (Friend erythroleukemia) cells before and after induction with DMSO (dimethyl sulfoxide). The results showed a dramatic decrease in the uptake of these molecules by induced cells amounting to a drop of about 70% of the control. Our experiments on 32P phosphate incorporation in these cells showed that newly synthesized globin mRNA appears in the cytoplasm about 10 hours after addition of DMSO. To determine whether the decrease in membrane permeability temporally coincided with globin mRNA induction, we measured the uptake of phosphate, leucine, etc at hourly intervals after addition of DMSO. A very interesting duality of effect was observed. At first hour time point, there was an increase in the uptake of these molecules and this increase continued up to about 8 hours. After that time the uptake showed first signs of decrease and this was almost concomitant to the initiation of induced globin mRNA synthesis. To further strengthen the interpretation of the uptake data, we used amphotericin B, which is known to increase membrane permeability. We found that if amphotericin B and DMSO are added together, the inducing effect of DMSO is totally wiped out, ie, no globin synthesis is observed. These results, therefore, strongly argue for a correlation between decrease in membrane permeability and induction of erythroid differentiation in FVC (Friend virus complex) transformed proerythroblasts. (no Refs)

MEMBRANE BIOGENESIS AND METABOLISM

63. EFFECTS OF MONENSIN ON MORPHOGENESIS AND INFECTIVITY OF FRIEND MURINE LEUKEMIA VIRUS.

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J Virol; 42(3):1067-1075 1982

The transport of the gp70 glycoprotein to the cell surface and concomitant release of infectious virus was inhibited by treatment of Friend murine leukemia virus-infected Eveline cells with the sodium ionophore monensin. Virus yields were reduced more than 50-fold by 10(-5) M monensin, whereas particle production was reduced by 50% in monensin-treated cells. The resulting particles failed to incorporate newly synthesized gp70 and p15(E), whereas the other structural proteins, p30, p15, p12, and p10, were incorporated into virions. However, monensin did not inhibit the incorporation into virions of preformed gp70. A reduction in the efficiency of cleavage of the PrENV glycoprotein precursor and a defect in the processing of simple endo-H-sensitive to complex endo-H-resistant oligosaccharides suggest that intracellular transport of gp70 may be blocked before its entry into the Golgi apparatus. Fewer particles were found to bud from the cell surface, but intracellular vacuoles with budding virions were detected. Ferritin labeling and pulse-chase studies suggested a cell surface origin for these vacuoles. These experiments indicate that monensin inhibits the transport of Friend murine leukemia virus glycoproteins at an early stage, with a resultant block in the assembly and release of infectious virus. (Author abstract) (37 Refs)

64. HERPESVIRUS GLYCOPROTEIN SYNTHESIS AND INSERTION INTO PLASMA MEMBRANES.

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J Virol; 42(2):678-690 1982

In the presence of the antibiotic tunicamycin (TM), glycosylation of herpes simplex virus glycoproteins is inhibited and non-glycosylated polypeptides analogous to the glycoproteins are synthesized. The synthesis of viral proteins and DNA occurs in TM-treated cells. By electron microscopy, nucleocapsids can be observed both in the nucleus and the cytoplasm and TM-treated cells; a small number of enveloped virions were observed on the cell surface. Analyses of the proteins in partially purified virus readily detects viral glycoproteins in the control cells, but neither glycoproteins nor nonglycosylated polypeptide analogs were observed in the virus prepared from TM-treated cells. By labeling the surface of infected cells with 125I, viral glycoproteins were detected as soon as 90 min after infection even when protein synthesis was inhibited with cycloheximide and glycosylation was blocked with TM. Labeling the proteins synthesized in infected cells with [35S]methionine showed that the surface glycoproteins detected in the cycloheximide- and TM-treated cells were not synthesized de novo after infection, but were placed on the cell surface by the infecting virus. Studies with metabolic inhibitors and a temperature-sensitive mutant blocked early in the infectious cycle showed that glycoproteins gA/gB and gD were synthesized soon after infection, but that the synthesis of gC was delayed. Under conditions of infection, in which gC and its precursor pgC are not produced, we have been able to observe the rela-

tionships between the glycosylated polypeptides that correspond to pgA/pgB and the nonglycosylated analog made in the presence of TM. (Author abstract) (47 Refs)

65. REGULATION OF CATABOLISM OF IGM HEAVY CHAINS IN A B LYMPHOMA CELL LINE.

Dulis BH, Kloppel TM, Grey HM, Kubo RT
Dept. Medicine, Natl. Jewish Hosp. and Res. Center, Denver, CO, 80206
J Biol Chem; 257(8):4369-4374 1982

The human lymphoma cell line Daudi has the phenotype of a nonsecreting B cell. This cell line synthesizes both the membrane and secreted forms of the IgM heavy chain, but only expresses functional membrane IgM. We have found that secreted type heavy chains (mu s) are rapidly degraded in these cells, with a half-life of 1.3 h. Some of the membrane type heavy chains (mu m) are also rapidly catabolized but some are expressed in a stable form with a half-life of 13 h. Inhibiting the initial glycosylation of heavy chains with tunicamycin has differential effects on the catabolic rates of mu s and mu m chains. The turnover of mu s chains is not affected by this inhibitor, but the degradation of mu m chains is much more rapid after tunicamycin treatment. In comparison with their glycosylated counterparts, nonglycosylated mu chains do not covalently assemble to a significant degree with light chains. Tunicamycin treatment of Daudi cells thus seems to inhibit formation of stable mu m protein, possibly by altering mu chain conformation and inhibiting its interaction with light chains. We conclude from these results that some mu m chains are specifically protected from proteolysis by posttranslational events. These processing events include covalent assembly with light chains, terminal glycosylation, and insertion into the plasma membrane. (Author abstract) (25 Refs)

66. IMMUNOGLOBULIN G BIOSYNTHESIS IN A HUMAN LYMPHOBLASTOID CELL LINE. DIFFERENCES BETWEEN MEMBRANE-BOUND AND SECRETORY FORMS OF GAMMA CHAINS.

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Eur J Biochem; 124(1):79-87 1982

The cultured human B lymphoblastoid cell line Maja synthesizes two forms of the gamma heavy chain of immunoglobulin G (IgG) that differ in apparent mol wt. The lower-mol-wt form is secreted into the culture medium as a water-soluble product in association with light chains and comigrates on dodecyl sulfate polyacrylamide gels with serum IgG gamma chains. The higher-molecular-wt form is not detected in culture supernatants. In distinction to the secreted form, the higher-mol-wt form is labeled by a lipophilic, photoactivatable nitrene and is inserted asymmetrically in a transmembrane orientation into rough microsomes. It is concluded that Maja cells synthesize secretory (gammas) and membrane-associated (gammam) forms of IgG heavy chains. Both forms of the gamma heavy chain are glycosylated, and can contain one or two asparagine-linked glycan units. The gammam and gammas heavy chains differ by about 10,000 in apparent mol wt. This difference resides exclusively in the polypeptide moiety. Although part of the difference comprises a transmembrane peptide and a cytoplasmic tail of apparent mol wt about 2,000 for gammam chains, a substantial segment of unique peptide is most probably present on the non-cytoplasmic side of the bilayer. The ionophore monensin inhibits the intracellular transport of gammas and gammam chains at a stage when they are sensitive to the enzyme endo-beta-N-acetylglucosaminidase H. In contrast, HLA-A and HLA-B antigens reach a stage at which they are insensitive to this enzyme in the presence of monensin, although their surface expression is inhibited by the ionophore. The implications of these results for the intracellular transport of membrane-associated glycoproteins are discussed. (Author abstract) (38 Refs)

67. DIFFERENT EXPRESSION OF LYT DIFFERENTIATION ANTIGENS AND CELL SURFACE GLYCOPROTEINS BY A MURINE T LYMPHOMA LINE AND ITS HIGHLY METASTATIC VARIANT.

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Eur J Immunol; 12(4):300-307 1982

Cloned lines of the methylcholanthrene-induced DBA/2 T lymphoma Eb and its highly metastatic variant line ESb were analyzed for differences in the expression of serologically detectable cell surface differentiation markers. Flow cytofluorographic analysis of cells stained with fluorescein isothiocyanate-conjugated monoclonal rat anti-mouse Thy-1, Lyt-1, Lyt-2 and complement-dependent cytotoxicity with mouse alloantisera against Lyt-3.2 and Ly-6.2 revealed, for the parental low metastasizing line, Eb, a phenotype of Thy-1+, Lyt-1-, Lyt-3+, Ly-6+, whereas the highly metastasizing variant line typed as Thy-1-, Lyt-1+, Lyt-2-, Lyt-3-, Lyt-6-. Analysis of galactose oxidase/NaB3H4-labeled glycoproteins from Eb and ESb clones by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate showed further phenotypic differences. Selective binding of radiolabeled glycoproteins to Helix pomatia or Vicia villosa-Sepharose, respectively, allowed the identification of T130 to be expressed on Eb cells and T145 to be expressed on some ESb clones. The latter antigen is expressed on murine cytotoxic T lymphocytes. Immune precipitation analysis revealed that Eb and ESb bear different molecular forms of the T200 antigen. Comparisons of iodinated surface proteins derived from tumor cells either treated or untreated with tunicamycin indicated that many of the differences in membrane proteins between Eb and ESb cells could be attributed to differences in glycosylation. Our results, derived from a defined tumor system of lymphoid origin, show that the progression from a low to a high malignant tumor line can be associated with changes in the expression of various defined cell surface differentiation antigens. The question of a possible relationship between tumor progression and cell differentiation or dedifferentiation is discussed. (Author abstract) (47 Refs)

68. EFFECTS OF PCB ON THE SYNTHESIS AND THE DEGRADATION OF PROTEINS COMPOSING MICROSOMAL MEMBRANES OF RAT LIVER.

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Trav Soc Pharm Montpellier; 41(1/2):141-142 1981

Synthesis and turnover of total proteins and isolated proteins (cytochrome B5 and cytochrome P450) in membranes of hepatic endoplasmic reticulum were studied in rats being fed polychlorobiphenyls (PCB). Rats receiving a regimen containing 20-100 ppm PCB showed a large increase (2-6x) in protein synthesis. Rats receiving low doses of PCB showed no change in the speed of protein degradation. (2 Refs) (In FRE)

69. REACTIVE INTERMEDIATES OF 2-ACETYLAMINOFLUORENE METABOLISM IN VITRO COVALENTLY LABEL SPECIFIC RAT LIVER MICROSOMAL PROTEINS.

Kaderbhai MA, Freedman RB, Bradshaw TK
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Adv Exp Med Biol; 136(partB):1183-1191 1982

Labeling of individual microsomal proteins during the metabolism of 2-acetylaminofluorene (AAF) in vitro was studied using electrophoretic techniques. After incubation of rat liver microsomes with (9-14C)AAF in the presence of NADPH, 3-5% of the added label was tightly bound to the membrane. Solubilization of this label by pronase and trypsin treatment indicated that the labeled derivative is covalently bound to microsomal protein. The binding increased linearly with time and was totally dependent on the presence of NADPH. With microsomes from 3-methylcholanthrene (MCA)-induced rats, binding was increased eight-fold compared to controls, but the increase was only two-fold with microsomes from phenobarbital (PB)-induced rats. These findings suggested that the reactive intermediate is generated by a cytochrome P-448-dependent reaction. Binding to microsomes was slightly enhanced by the presence of cytosolic proteins. The incorporation of label was mainly in a few protein bands. Mol wt of these bands were close to those of cytochrome P-450, cytochrome b5, and their reductases. Although the extent of labeling of these proteins increased with time of reaction, the pattern of labeling was not altered. With microsomes from MCA-induced rats, the pattern was similar to that with control microsomes, but with microsomes from PB-induced rats only a single band was labeled significantly corresponding to a PB-induced cytochrome P-450 apoprotein. With microsomes from rats pretreated with AAF, the pattern of labeling was similar to that with controls and MCA-induced rats. Extensive labeling of a band specifically induced by AAF pretreatment was observed. The band was identified as epoxide hydratase. (no Refs)

70. GENERATION OF PROCOAGULANT ACTIVITY (PCA) BY PHORBOL-ESTERS-INDUCED MACROPHAGES DERIVED FROM A LEUKEMIC PROMYELOCYTIC CELL LINE (HL-60).

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Blood; 59(5):1061-1066 1982

Normal human monocytes and macrophages, as well as leukemic promyelocytes, generate potent procoagulant activity (PCA) resembling thromboplastin. In the present study only mild PCA was detected in a leukemic promyelocytic cell line (HL-60) and in promyelocytic cells induced to differentiate into neutrophils by dimethylsulfoxide (DMSO). After exposure to 12-O-tetradecanoylphorbol-13-acetate (TPA), the leukemic promyelocytic cells assume characteristics specific to monocytes and macrophages. This differentiation was associated with potent PCA generation. No PCA was found in lymphoblasts and several lymphoid cell lines after exposure to TPA. The PCA in TPA-induced HL-60 cells resembles tissue thromboplastin and normal monocyte PCA in several aspects: (A) All three accelerate clotting through the extrinsic coagulation pathway; (B) all three are inhibited by concanavalin-A and protected by methyl-alpha-D-mannopyranoside, suggesting that the PCA contains carbohydrate moieties; (C) the PCA is located in the cell membrane. However, the mechanism of PCA generation in normal monocytes and in TPA-induced HL-60 cells differs in several aspects: (A) Endotoxin and phytohemagglutinin, which are potent stimuli of PCA generation in normal monocytes, have no effect on TPA-induced HL-60 cells; (B) HL-60 cells treated with TPA develop PCA before acquiring any other specific characteristic of monocyte or macrophages; (C) the development of PCA in TPA-induced macrophages is not dependent on cell adhesion or the presence of T cells. In spite of these difference, the production of PCA is additional evidence of the similarity between TPA-induced macrophages and normal human monocytes and macrophages. The HL-60 cells may provide a unique model for studying PCA generation in mononuclear cells. (Author abstract) (29 Refs)

71. TPA INDUCED ANTIGENIC MODULATIONS ON A HUMAN LEUKEMIC CELL LINE: REH (MEETING ABSTRACT)

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VI Meeting of the European Association for Cancer Research, October 12-15, 1981, Budapest, Hungary. The European Association for Cancer Research 275 pp., 1981.

It has been shown by Castagna et al that 12-O-tetradecanoyl phorbol-13 acetate (TPA) induces substrate adhesion, growth inhibition and decrease of membrane microviscosity in a non T non B acute lymphoblastic leukemia cell line REH. In this study we have found that those effects were associated with other phenotypic changes. FACS (Fluorescence-activated cell sorter) analysis showed firstly time dependent differences in the repartion of cellular scatter measurement of size between control and TPA treated cells, especially in the adherent cells. No B cell, T cell or monocytic characteristics appeared on the cells (E rosettes SIg, OKT3,4,6,8) but FACS analysis showed variations in the expression of HLA-DR and cALL antigens different from those spontaneously observed during normal cell growth. These observations were coupled with cell cycle studies using acridine orange. The results will be discussed according to several aspects including differentiation, proliferation and physicochemical characteristics of cell membranes. (no Refs)

72. INTERCELLULAR STRUCTURE FORMATION AND ALTERATION OF GLYCOPROTEIN METABOLISM INDUCED BY 5-HEXYL-2' DEOXYURIDINE /HUDR/ (MEETING ABSTRACT)

Lapis K, Jeney A, Timar J, Ecsedi G, Hidvegi E, Kopper L
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Proc Am Assoc Cancer Res; 23:858 1982

HUdR, showed no action on thymidine-kinase, thymidylate-synthetase and did not incorporate into DNA, although it inhibited the growth of Ehrlich ascites tumor treated ip. Since electron microscopic studies revealed the formation of intercellular structures (ie tight junctions in 10% of the cells) and also the defect in Concanavalin A binding to the cell surface, HUdR action on glycoprotein synthesis via nucleoside transport system was assumed. This was confirmed by demonstrating a 50% inhibition of 3H-glucosamine incorporation after in vivo HUdR treatment (100 mg/kg ip 3 hours) and also by detecting the preferential uptake of 14C-HUdR into the Golgi apparatus (9x10(4) DPM per mg protein) and plasma membrane (1.7x10(4) DPM per mg protein) relative to whole cell (1x10(4) DPM per mg protein). Galactosyltransferase in cell free system was inhibited at 6.8x10-5M of HUdR by 50%. These results

seem to indicate that HUdR is a pyrimidine antimetabolite inhibiting cell proliferation by interfering with glycoprotein biosynthesis and at the same time intercellular structures are formed in dedifferentiated Ehrlich ascites tumor cells. (1 Ref)

CELL ADHESION

73. PHENOMENON OF HUMAN T CELLS ROSETTING WITH SHEEP ERYTHROCYTES ANALYZED WITH MONOCLONAL ANTIBODIES: MODULATION OF A PARTIALLY HIDDEN EPITOPE DETERMINING THE CONDITIONS OF INTERACTION BETWEEN T CELLS AND ERYTHROCYTES.

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J Exp Med; 155(5):1317-1333 1982

The monoclonal antibody (MAb) anti-D66, which identifies an epitope that appears to be involved in E-rosette formation, is described. Unlike MAb 9.6, anti-D66 does not block rosettes when erythrocytes have been pretreated with neuraminidase or 2-aminoethylisothiouronium bromide, although it blocks E-rosettes made at both 4 C and 37 C. Cell surface molecules from thymocytes were labeled and isolated by immunoprecipitation and acrylamide gel electrophoresis. D66 and 9.6 epitopes were carried by the same molecule, and although 9.6 epitopes were readily exposed on the cell surface, a large fraction of the D66 epitopes was covered by the glycocalixte, from which it can be unmasked by anti-D66 sensitization plus anti-mouse immunoglobins. D66 is an epitope involved in the interactions between T lymphocytes and erythrocytes, and is clearly different from the epitope defined by MAb 9.6. A striking correlation was observed between the surface density of D66 in a given T cell population and its ability to form stable rosettes at 37 C. (48 Refs)

74. FIBRONECTIN DISTRIBUTION PATTERN IN SOLID HUMAN TUMOURS (MEETING ABSTRACT)

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VI Meeting of the European Association for Cancer Research, October 12-15, 1981, Budapest, Hungary. The European Association for Cancer Research 275 pp., 1981.

Fibronectin, a structural glycoprotein, present on cell membranes and in the intercellular matrix has been reported to be lost in numerous transformed cells in cultures. Using indirect immunofluorescence, its distribution pattern has been studied in a variety of solid human tumors and corresponding normal tissues. Fibronectin could be detected on normal epithelial cell membranes, in periglandular and perivascular basement membranes and in the intercellular stroma. No modification of this distribution pattern was detected in a variety of benign tumors or in certain pathological non-maiignant disorders. In infiltrating mammary carcinomas, cell membrane staining disappeared even before invasion. The staining pattern of the glandular basement membranes became patchy, discontinuous and finally disappeared with invasion. Similar modified fibronectin patterns were observed in bronchial, endometrial, prostatic, urinary bladder and thyroid carcinomas. In sarcomas, fibronectin disappeared from the membranes of transformed cells and decreased in intensity in the intercellular matrix. (no Refs)

75. THE TRANSFORMATION-SENSITIVE GLYCOPROTEIN LAMININ IS AN AGGLUTININ (MEETING ABSTRACT)

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Membrane Biochemistry Section, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20205
Med Pediatr Oncol; 10(1):100 1982

Laminin is a high molecular weight glycoprotein involved in the adhesion of a variety of epithelial cells to type IV collagen of basement membranes. The protein consists of at least two polypeptide chains of 220,000 and 440,000 daltons joined to each other by disulfide bonds. Laminin is found to agglutinate formalinized sheep erythrocytes at protein concentrations of under 1 ug/ml. This finding provides a simple model system for analyzing laminin-mediated adhesion and for searching for its cell surface receptor in competitive inhibition experiments. Laminin-mediated hemagglutination is markedly inhibited by highly negatively charged phospholipids and by purified solubilized gangliosides. The inhibition is dose-dependent and competitive; hemagglutination by 4 ug/ml laminin is half-maximally inhibited by less than 1 uM phosphatidic acid and by less than 5 uM gangliosides. The order of inhibition by gangliosides is GD1a greater than GT1b greater than GM1. A series of other lipids, ganglioside constituents, monosaccharides, glycosaminoglycans, and glycoproteins are either less effective or without detectable competitive inhibitory activity. These results suggest that hypothesis that laminin mediates normal epithelial cell adhesion by binding to a cell-surface receptor that may contain a negatively charged cell-surface component such as a ganglioside or another highly charged lipid. (no Refs)

OTHER RELATED STUDIES

76. REGULATORY ROLE OF MEMBRANES IN TUMOR GROWTH.

Burlakova EB, Pal'mina NP
Inst. Chemical Physics, Moscow, USSR
Vestn Akad Med Nauk SSSR; (3):74-86 1982

Biological functions of cell membranes are discussed with special emphasis on the role of membranes in the regulation of cell metabolism. Neoplastic transformation was shown to be associated with the changes in the structure and function of cell membranes. Changes in the protein, glycolipid, and sialic acid composition of the cell membranes were observed in a number of tumors. Characteristic features of tumor cell membrane include the increase of sphingomyelin concentration and the presence of cardiolipin. Recent studies showed that lipid dedifferentiation of the membranes of tumor cells can not be considered as the tumor-specific trait. (141 Refs) (In RUS)

77. IN VITRO DEGRADATION OF RADIOLABELLED, INTACT BASEMENT MEMBRANE MEDIATED BY CELLULAR PLASMINOGEN ACTIVATOR.

Sheela S, Barrett JC
(c/o Barrett) Lab. Pulmonary Function and Toxicology, Natl. Inst. Environmental Health Sciences, Research Triangle Park, NC, 27709
Carcinogenesis; 3(4):363-369 1982

A simple and unique procedure for the isolation of intact basement membrane from Syrian hamster lung was developed. The method involved an initial 24 hr extraction of minced lungs with 0.3 M acetic acid followed by treatment with N-

lauroyl sarcosine, an anionic detergent. The tissue was washed several times in 0.85% NaCl and subjected to deoxyribonuclease treatment followed by washings with 0.85% NaCl and distilled water. The residue was shown to be basement membrane by electron microscopy and by amino acid analysis. The basement membrane was radiolabeled by reductive alkylation and 80-90% of the radiolabel was present in the glycoprotein component of the basement membrane. A simple assay to measure degradation of [3H]basement membrane was developed. When added to growing tumor cells in the presence of growth medium and serum, the [3H]basement membrane was solubilized extensively. The reaction was linear for 24 hr at which time up to 90% of the labeled material had been released. In contrast, 5% of the label was solubilized in medium plus serum alone or in the presence of normal Syrian hamster embryo cells. The solubilization of the [3H]basement membrane was primarily due to degradation of the glycoproteins of the basement membrane as shown by Sephadex G-200 gel chromatography. The abilities of the tumor cells to degrade the [3H]basement membrane correlated with their fibrinolytic activity and inhibitors of plasmin inhibited the reaction. The activity of the cells in this assay was dependent upon the presence of plasminogen in the medium. These results indicate a role for plasminogen activator in the pathogenesis of invasive tumor cells. (32 Refs)

78. CELL SURFACE PROPERTIES, PROTEASE OUTPUT AND METASTATIC POTENTIAL OF HUMAN AND ANIMAL MAMMARY TUMOURS (MEETING ABSTRACT)

Tarin D
Histopathology Department, John Radcliffe Hospital, Oxford, England
VI Meeting of the European Association for Cancer Research, October 12-15, 1981, Budapest, Hungary. The European Association for Cancer Research 275 pp., 1981.

Tumors differ very much from one another in their ability to spread and establish colonies in distant organs even when inoculated directly into the bloodstream. Our experiments indicate that these differences in behavior are attributable, at least in part, to intrinsic properties of the cells from different tumors. For a given tumor the degree of pulmonary colonization after intravenous inoculation is similar whichever syngeneic animal is inoculated. We have also found that naturally-occurring murine mammary tumors which can colonize the lungs after intravenous inoculation have significantly greater output of collagenase than low colonizers or non-neoplastic tissue. Using lectin agglutinability with Concanavalin-A and Wheat germ agglutinin as a means of examining surface properties, we have found no consistent differences between tumors of high and low pulmonary colonization potential, but it has been observed that cells from secondary deposits in the lung are reproducibly less agglutinable than cells in the primary tumors from which they arise. The implications of these findings are that these markers may be useful for assessing tumor metastatic potential in humans. Similar studies are, therefore, in progress on human tumors. (no Refs)

79. ABILITY OF TUMOR CELLS TO RESIST HUMORAL VS. CELL-MEDIATED IMMUNE ATTACK IS CONTROLLED BY DIFFERENT MEMBRANE PHYSICAL PROPERTIES.

Schlager SI
Dept. Microbiology, Univ. Notre Dame, Notre Dame, IN, 46556
Biochem Biophys Res Commun; 106(1):58-64 1982

Treatment of P815 murine mastocytoma cells with adriamycin increased their susceptibility to killing by antibody + complement (Ab-C) compared to controls; mitomycin C did not show this effect. Cells washed free of the drug and incubated in the drug-free medium regained control levels of susceptibility to Ab-C. Opposite effects of these two drugs on the susceptibility of P815 cells to killing by cytotoxic T-lymphocytes (CTL) were observed, ie, mitomycin C, but not adriamycin, increased the susceptibility to CTL killing. Cells washed free of drug and reincubated in drug-free medium reverted to control levels of susceptibility to CTL. P815 cells cultured with hydrocortisone were rendered more resistant, compared to controls, to Ab-C or CTL killing; epinephrine treatment had no effect on the susceptibility to either Ab-C or CTL attack. Cells washed free of hormone again reverted to control levels of susceptibility to Ab-C and CTL. Membranes from adriamycin-treated cells exhibited increased membrane fluidity compared to controls; mitomycin C had no effect. Hydrocortisone treatment decreases membrane fluidity while epinephrine had no effect. Mitomycin C decreased, and hydrocortisone increased, the net negative cell surface charge density. Adriamycin and epinephrine had no effect. Drug- or hormone-treated reverted cells were similar to control cells in membrane fluidity and surface charge. These results indicate that plasma membrane fluidity and cell surface charge are unique physical properties that control the susceptibility of tumor cells to Ab-C and CTL killing, respectively. (19 Refs)

80. MEMBRANE CARBOHYDRATE ANALOGUES WITH ANTITUMOR ACTIVITY (MEETING ABSTRACT)

Bernacki RJ, Sharma M, Angelino N, Korytnyk W
Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, NY, 14263
Proc Am Assoc Cancer Res; 23:803 1982

We have synthesized several carbohydrate analogues as potential inhibitors or modifiers of cell surface glycoconjugates. These compounds have been tested as antitumor agents in a number of in vitro and in vivo test systems. Two agents, a galactosamine and a mannosamine analogue, have demonstrated significant biological activity. 2-Acetamido-2,4-dideoxy-4-fluoro-1,3,6-tri-O-acetyl galactopyranose, a lipophilic N-acetylgalactosamine analog designed to inhibit carbohydrate chain elongation, inhibited L1210 leukemia cell growth by 50% in vitro at 3.5 x 10-5M. It specifically inhibited the incorporation of D-mannose and L-leucine into L1210 cells at these concentrations. Administration of 50 or 100 mg/kg/day x 5 ip to DBA/2Ha mice with L1210 leukemia increased their life span by over 50%. 2-Amino-2-deoxy-N'-methyl-N'-nitrosoureido-1,3,4,6 tetra-O-acetyl-D-mannopyran ose, a lipophilic mannosamine analogue related to streptozotocin, was found to inhibit L1210 cell thymidine incorporation more specifically than sugar incorporation. It inhibited 50% of L1210 cell growth in vitro at 5 x 10-5M. This compound at 30 mg/kg/day x 5 ip cured DBA/2Ha mice with L1210 leukemia and at 50 mg/kg/day x 5 increased the life span of DBA/2J mice by 50%. The effects of both these agents on plasma membrane glycoconjugate structure and formation and their effects on other metastatic tumors in mice are currently being investigated. (no Refs)

81. IN 'UNDIFFERENTIATED' PC12 CELLS, GERL IS NOT SEGREGATED FROM THE GOLGI APPARATUS.

Burchanowski BJ, Hogue-Angeletti R, Stieber A, Gonatas J, Gonatas NK
Dept. Pathology, Univ. Pennsylvania Sch. Medicine, Philadelphia, PA, 19104
J Neurocytol; 11(2):323-333 1982

Endocytosis of conjugates of horseradish peroxidase (HRP) with ricin and wheat germ agglutinin (WGA) was studied in rat adrenal pheochromocytoma cells (line PC12) cultured in the absence of nerve growth factor (NGF). Activity of acid phosphatase (AP) was not confined to a single cisterna and vesicles at the transaspect (mature face) of the Golgi apparatus corresponding to the Golgi-endoplasmic reticulum-lysosome (GERL) of other cell types. In AP preparations, the reaction product was found in GERL and in several cisternae of the Golgi apparatus proper. AP activity in the parallel Golgi cisternae was often discontinuous. Large AP-positive dense bodies were seen throughout the cytoplasm, small ones were confined to the Golgi region. With ricin-HRP and WGA-HRP, following endocytosis of peroxidase-labeled lectin, reaction product was found in vesicles, tubules, and dense or residual bodies throughout the cytoplasm. Large regions of anastomosing tubules contained dense labeling and large vacuoles contained only peripheral stain. No peroxidase reaction product was observed in the parallel cisternae of the Golgi apparatus, but labeling was seen within vesicles and tubules near the Golgi apparatus. Only dense bodies were seen in cells incubated in free HRP. In PC12 cells grown without NGF, GERL is apparently not segregated from the Golgi apparatus by either AP cytochemistry or by the functional criterion of endocytosis of lectin-HRP conjugates. (27 Refs)

82. PHORBOL ESTER INDUCTION OF LEUKEMIC CELL DIFFERENTIATION IS A MEMBRANE-MEDIATED PROCESS.

Cooper RA, Braunwald AD, Kuo AL
Hematology-Oncology Section, Dept. Medicine, Cancer Center, Hosp. Univ. Pennsylvania, Philadelphia, PA, 19104
Proc Natl Acad Sci USA; 79(9):2865-2869 1982

Phorbol esters are potent inducers of macrophage-like differentiation in the HL-60 promyelocytic leukemia cell line. The sequence of events by which they bring about this transition is poorly understood. However, it is known that phorbol esters bind to the surface membrane of HL-60 cells and to various other cells as well. Our studies were directed toward determining the biologic importance of this membrane association. [3H]Phorbol dibutyrate (PBu2) was specifically bound by HL-60 cells with a Kd of 23 nM and with 1.9 x 10(5) binding sites for [3H]PBu2 per cell. There was no internalization of bound [3H]PBu2. Specific binding was fully reversible upon washing in fresh medium, and [3H]PBu2 added thereafter bound normally to its receptor. Within 10 min of binding, PBu2 stimulated [14C]choline incorporation into phosphatidylcholine, with a rapid return to normal upon removal of the PBu2. Membrane-bound PBu2 progressively inhibited DNA synthesis, with 70% inhibition by 8 hr. This process was interrupted if the PBu2 was removed and little recovery of DNA synthesis occurred in previously inhibited cells. Between 8 and 16 hr, PBu2 induced adherence of cells to plastic, but only in those cells in which phosphatidylcholine synthesis was stimulated, and this process was also interrupted if PBu2 was removed prior to 16 hr. Similarly, nonspecific esterase, which develops after 72 hr of incubation, was induced in cells exposed to PBu2 for the initial 16 hr but not in cells exposed for 5 hr. These studies demonstrate that phorbol esters exert their effects while retained at the cell surface. Inhibition of cell growth and the acquisition of surface and enzymatic properties that characterize macrophages are separable events, even of which proceeds through a receptor-mediated, transmembrane process. The stimulation of phosphatidylcholine synthesis appears to be a part of that process. (Author abstract) (34 Refs)

83. EFFECT OF PULSE-PERIODIC LASER UV IRRADIATION OF LOW INTENSITY ON PROLIFERATING AND RESTING TUMOR CELLS.

Karu TI, Kalendo GS, Letokhov VS, Lobko VV
Res. Center Technological Lasers, Moscow, USSR
Dokl Akad Nauk SSSR; 262(6):1498-1501 1982

Irradiation of HeLa cells in the logarithmic growth phase (proliferating cells) or in the stationary phase (resting cells) with low intensity pulse UV laser produced decrease of DNA synthesis permeability of cell membrane in proliferating cells. In the resting cells, pulse laser irradiation stimulated DNA synthesis and increased permeability of cell membranes for 3H-thymidine. Irradiation with continuous lasers inhibited DNA synthesis in proliferating cells but did not affect DNA synthesis in resting cells. (4 Refs) (In RUS)

84. SIMULTANEOUS DETERMINATION OF T-ANTIGEN AND ABO CELL SURFACE ANTIGENS IN TRANSITIONAL CELL CARCINOMA (MEETING ABSTRACT)

Kidd D, Javadpour N, Fourcroy J, O'Connel K
No affiliation given
77th Annual Meeting of the American Urological Association, Inc., May 16-20, 1982, Kansas City, Missouri. American Urological Association 254 pp., 1982.

The purpose of this study was to investigate the changes in cell surface antigens which result in the interruption of communication and interaction through the cell surface membrane. These changes reflect an alteration in the genome which modifies the biosynthetic pathways of the Golgi apparatus. The demonstration of T-antigen expression in breast carcinoma and the loss of blood group iso-antigens in invasive transitional cell carcinoma prompted inquiry into the correlation between the carbohydrate precursor of the human blood system MN and the epithelial surface blood group isoantigens. A red cell adherence assay to detect T-antigen on deparafinized slides of transitional cell carcinoma of the bladder was developed. Peanut agglutinin which has anti-T properties as well as other nonspecific binding sites is layered over the slides, washed, then covered with neuraminidase treated red cells. Red cell adherence was noted on areas of the bladder tumor that binds plant lectin. This was compared with the specific red cell adherence (SRCA) for ABO antigen on the same tissue samples. Of the 17 patients, 14 demonstrated loss of their cell surface ABO antigens. Of the 14, 28% demonstrated expression of the T-antigen. This would imply that T-antigens may be complementary to A, B, O, Ag in predicting potential malignancies of bladder cancer when the conventional histologic examination can not provide any prediction. (no Refs)

85. INHIBITORY EFFECTS OF VARIOUS OXYGENATED STEROLS ON THE DIFFERENTIATION AND FUNCTION OF TUMOR-SPECIFIC CYTOTOXIC T LYMPHOCYTES.

Spengrude GJ, Sherris D, Daynes RA
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Transplantation; 33(5):482-491 1982

These studies were undertaken to determine the effect of various oxygenated sterols (representing a number of known cholesterol-derived photoproducts) on the generation (afferent) and function (efferent) of cytotoxic T lymphocytes (CTLs). Cell-mediated immune responses which result in the generation of both alloantigen-specific and syngeneic tumor-specific CTLs were evaluated. The results of our studies established the following points: (1) the afferent phase of an immune response is particularly sensitive to the effects of some cholesterol photoproducts while other oxidized sterol derivatives, known to exist in skin following UV light (UVL) exposure, have minimal influence on the generation of CTL responses; (2) the only known carcinogenic photoproduct of cholesterol (cholesterol alpha-oxide) is a very weak inhibitor of lymphocyte function; and (3) presentation of oxygenated sterols via liposome membranes is 10-fold more effective than their administration in soluble form; (4) 25-hydroxycholesterol and 20alpha-hydroxycholesterol were found to be the most potent inhibitors, causing a parallel depression in lymphocyte proliferation, CTL activity, and endogenous sterol biosynthesis, and (5) 7beta- and 7alpha-hydroxycholesterol functioned in a unique manner, causing an abrogation of CTL activity without depressing proliferative responses and endogenous sterol biosynthesis to a significant degree. These studies represent the first time that an attempt has been made to evaluate a possible biochemical basis for immune suppression known to precede experimental UVL carcinogenesis. Our results suggest that some, but not all, of the oxidation products of cholesterol found in skin following UVL irradiation exert an influence on immune responses. Derivatives of cholesterol could be partially responsible for the alterations in immunological potential of the UVL-exposed host. The induced presence of an inhibitor of CTL differentiation or function at a time following UVL tumor emergence may play a role in this process by providing an early tumor escape mechanism. (37 Refs)

86. PHORESIS OF TUMOR CELLS.

Shishido H
Third Dept. Internal Medicine, Dokkyo Medical Univ., Saitama Prefecture, Japan
Nippon Rinsho; 39(8):2856-2861 1981

By measuring migration speed and cell surface changes in cell electrophoresis, properties of membrane surface and membrane phenomena can be studied. From a histogram of the migration speed of peripheral blood cells obtained from 50 healthy adults, 3 peaks,-0.75+-0.09 (S),-0.89+-0.17 (F), and-1.17+-0.06 (R) micro/sec/v/cm were obtained. In various non-cancerous hematopoietic diseases, when leukocytes were electrophoresed, F peak increased in lymphocytosis, whereas S peak increased in neutrophilia. In contrast, in acute leukemia, abnormal peaks, different from S and F peaks, are noted. A similar abnormal peak was obtained in acute myelocytic leukemia, acute lymphocytic leukemia, acute monocytic leukemia, and acute premyelocytic leukemia, despite their morphological differences. In chronic myelocytic leukemia, a tendency to disperse toward high speed from normal S peak was clearly seen; and an abnormal peak similar to that of other acute leukemia was also seen. Normal lymphocytes showed in F peak with T cells faster migration speed than B-cells. Lymphocytes from various leukemia clearly exhibited abnormal peak, suggesting that electrophoretic properties may serve as malignancy index and as a tool for studying malignancy. These abnormal peaks rapidly decreased as therapy progressed. Therefore, electrophoretic mobility can also be used to judge the therapeutic effect. (28 Refs) (In JPN)

87. LIPOSOME-MEDIATED IMMUNOGENICITY OF HUMAN COLON TUMOR ANTIGENS (MEETING ABSTRACT)

Rutzky LP, Goodwin TJ, Sengupta J, Kahan BD, Tom BH
University of Texas Medical School, Houston, TX, 77030
Fed Proc; 41(3):791 1982

Liposomes bearing human tumor membrane (MN) vesicles were used to immunize rabbits. In contrast to the broad specificity of whole cell immunization, liposome-borne MN antigen (Ag) immunization resulted in greater specificity toward colon cancer than to either breast or bladder tumor cells. Multilamellar liposomes (MLV) at 7:2:1 molar ratios of phosphatidyl choline, cholesterol and phosphatidic acid were prepared by the method of Bangham. MLV membrane Ag complexes were made with sonicated membrane isolated from LS174T colon tumor cells. MLV membranes prepared together with MN (ie, MN-MLV antigens) or MN added to preformed MLV (ie, MN+MLV antigens), and MN antigens alone were used as immunogens. Rabbits were immunized iv with 100 ug protein of each antigen group. Boosters (iv) were at days 13 and 29. Binding assays were performed by indirect radio immunoassay on viable tumor cell targets. Peaks of antibody titers occurred on days 20 and 35. Although titration of the 3 groups of antisera on days 9, 20 and 35 indicated no unique relationship between Ag groups and titers, the MN+MLV group was distinguished by earlier reactivity and greater specificity. The amount of tumor Ag exposed on the MN+MLV was 75-80% to 57% for MN-MLV groups. Whether this difference in Ag density resulted in greater immunogenicity of the MN+MLV Ag needs to be evaluated. Thus liposomes are effective immunogenic carriers of human tumor Ags. (no Refs)

88. PROPERTIES OF SJL STRAIN LYMPHOMAS WHICH FAIL TO EXPRESS MEMBRANE H-2DS (MEETING ABSTRACT)

Kuhn M, Dyer CA, Mayo L, Jagdish Babu K, Righthand VF, Lightbody J, Beisel KW, Lerman SP
Wayne State University, Detroit, MI, 48201
Fed Proc; 41(3):410 1982

Correlation was sought between alterations in expression of MHC antigens and biological properties of long and short term transplantable lymphomas (reticulum cell sarcomas) in SJL mice. As determined by microcytotoxicity dye exclusion and confirmed by immunoprecipitation using Staph protein A, a progressive loss of membrane H-2Ds was noted upon short term sequential transplantation. H-2Ks expression was normal. Those tumors which lost expression of H-2Ds transplanted with fewer cells and caused greater increases in lymphoid tissue weights. Tumor cells were freed of contaminating host cells by growth in (C57BL/10 x SJL/J)F1 mice and treatment with alloantisera and C. Spontaneous tumor bearers were evaluated for their response to radiotherapy and respective tumor cells were studied for radiation sensitivity and for their requirement for radiation sensitive normal host cells for growth. Capacities of tumor cells to recirculate and to induce an in vitro proliferative response in syngeneic lymphoid cells were studied as was the ability of tumorous lymphoid cells to synthesize in-

terferon-like-activity. It is suggested that tumor cells which lose H-2Ds may be at a growth advantage over tumor cells with a normal H-2 antigen display. (no Refs)

89. MODIFIED SPONTANEOUS CELL-MEDIATED CYTOTOXICITY (SCMC) BY ALLOGENEIC GLIOMA MEMBRANES AND THE CHEMICAL DERIVATIVE (MEETING ABSTRACT)

Zanker KS
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Proc Am Assoc Cancer Res; 23:1065 1982

A proportion of human blood lymphocytes, recently characterized as large granular lymphocytes, express SCMC potential against a wide variety of in-vitro grown tumor target cells. Peripheral blood lymphoid cells (PBL) from human donors with no known immunization history to tumors were freshly prepared by Ficoll centrifugation. A glioma cell line, established from an astrocytoma II, located in the right hemishere, was raised and the tumorigenicity of the cells proved at the 10th passage rate by inoculation of 2x10(6) cells in nu/nu Balb/c mice. Short term exposure (4 hr) of PBL in-vitro to 14C-nicotinamide labelled glioma cells exhibited a SCMC of 29% at an effector:target cell ratio of 25:1. The membrane preparation was checked for plasma membranes by marker enzymes and electron microscopy. A number of 2x10(6) PBL was exposed for 12 hr to about 500ug/ml enriched plasma membranes, quantitated as soluble membrane proteins. SCMC capacity increased in a series of nine experiments to the mean of 48% in comparison to unstimulated PBL. In a series of five experiments, freshly prepared glioma membranes were chemicaly modified with 2,4,6-Trinitrobenzene-sulfonate and likewise used to stumulated PBL. The SCMC capacity of these set of PBL was shifted to 67%. The glioma cell membrane derivatives were subjected to Folch extraction and the upper and lower phase of the Folch solution were analyzed for phenylated constituents. Mainly amino groups of proteins and phosphatidylethanolamine were targets for the modifying agent. (no Refs)

90. MEMBRANE MICROVISCOSITY INCREASES IN REGRESSING NITROSOMETHYLUREA (NMU) INDUCED MAMMARY CARCINOMA (MEETING ABSTRACT)

Dave JR, Knazek RA, Liu SC
Laboratory of Pathophysiology, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20205
Proc Am Assoc Cancer Res; 23:938 1982

The objective of these studies was to determine if the changes observed in prolactin-binding capacity of membranes from growing and regressing NMU induced mammary carcinoma correlated with those observed in membrane microviscosity measurements. Buffalo female rats were intravenously injected with 5 mg/100 g body weight of NMU at 50, 80 and 110 days of age. Mammary tumors induced in Buffalo rats by treatment with NMU are known to regress after oophorectomy. These rats were, therefore, either sham operated or oophorectomized at 5 months of age and sacrificed at 1, 3, 4, 5 and 8 days after the surgery. The 100,000 x g membrane pellets of these growing or regressing tumors were used for oPRL binding and fluidity studies. Membrane microviscosity was studied by fluorescence polarization at 24 C using a lipid probe 1,6 diphenylhexatriene (1 uM in PBS at pH 7.1). A 46% decrease in prolactin-binding capacity was observed in membranes obtained from animals sacrificed 8 days after oophorectomy. Such a decrease in prolactin binding capacity of membranes from regressing tumors is well documented. Fluorescence polarization values were low in membranes from growing tumors which increased progressively with time after oophorectomy, reaching a maximal value at 5 days after oophorectomy, the values being 0.140, 0.188, 0.226, 0.227, 0.248 and 0.250, for membranes from growing tumors and 1, 3, 4, 5 and 8 days regressing tumors, respectively. A similar difference in membrane fluorescence polarization values was observed in growing and regressing DMBA-induced mammary carcinoma. (no Refs)

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STRUCTURAL AND FUNCTIONAL ASPECTS OF CELL MEMBRANES

SERIES CB06 No. 82/11

NOVEMBER, 1982

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. IMMUNOFLUORESCENT DETECTION OF ERYTHROCYTE SIALOGLYCOPROTEIN ANTIGENS ON MURINE ERYTHROID CELLS.

Sarris AH, Palade GE
Section Cell Biology, Yale Univ. Sch. Medicine, New Haven, CT, 06510
J Cell Biol; 93(3):591-603 1982

A sialoglycoprotein fraction isolated from murine (DBA/2) erythrocytic ghosts was used to raise antibodies in rabbits. By immune-IgG (serum)-[125I]protein A overlays, the antibodies were found to react positively with the four sialoglycoprotein monomers (gp-2.1, gp-2.2, gp-3.1 and gp-3.2) of the original fraction, with the sialoglycoproteins detected in erythrocytic ghosts (gp-2.1 and gp-3.1), with a diffuse component (probably a macroglycolipid) trailing around gp-3.1 in SDS polyacrylamide gel electrophoretograms of solubilized ghosts, and with a minor sialoglycoprotein hidden under this trail. IgG's isolated from immune and nonimmune rabbit sera were conjugated to tetramethylrhodamine isothiocyanate and used to survey, by

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fluorescence microscopy, the distribution of the cognate antigens on the three different erythroid lines known to succeed each other during the life span of the mouse. In the peripheral blood of the adult, the antibodies recognized only mature erythrocytes; they did not crossreact with either platelets, monocytes, or different types of granulocytes. In the spleen of adult anemic mice, the antibodies reacted weakly with proerythroblasts and strongly with all types of erythroblasts. In enucleating erythroblasts, antigens were preferentially segregated on the cell membrane of the nascent reticulocyte. In the 10-day-old embryo, antigens were already present on the primitive nucleated erythrocytes (produced by the blood islets of the yolk sac), and in the 14-d fetus they were found on all hepatic erythroblasts and derived non-nucleated erythrocytes. A positive immunoreaction was also obtained on Friend erythroleukemic cells, before or after induction by dimethyl sulfoxide. Nonimmune serum, or nonimmune IgGs gave negative reactions in all cases. The antibodies were species-specific: ey did not crossreact with either human or rat erythrocytes. (Author abstract) (70 Refs)

2. CELL SURFACE GLYCOPROTEIN AND ASPARAGINE-LINKED SUGAR CHAIN PATTERNS OF RAT ERYTHROLEUKEMIC CELL LINES.

Shiraishi N, Yoshima H, Maeda S, Mizoguchi A, Matsumoto A, Sugiyama T, Kobata A
(c/o Kobata) Dept. Biochemistry, Kobe Univ. Sch. Medicine, Chuo-ku, Kobe, Japan
Cancer Res; 42(7):2884-2893 1982

In order to elucidate whether chromosomal change or induction of differentiation is correlated with the change of the patterns of cell surface glycoproteins and asparagine-linked sugar chains, glycoproteins and carbohydrates of five rat erythroleukemic cell lines (K1DB, D5A1, K2D, K2DA, and K4D) were studied by sodium dodecyl sulfate:polyacrylamide gel electrophoresis and hydrazinolysis followed by gel chromatography, respectively. The surface glycoprotein patterns of five cell lines were very similar, each containing four glycoproteins with molecular weights of 250,000, 115,000, 80,000, and 28,000, respectively. Only D5A1, which can be induced to erythroid differentiation, had two additional glycoproteins, with molecular weights of 135,000 and 65,000, respectively. All neutral oligosaccharides obtained from plasma membranes of five cell lines by hydrazinolysis were of the high-mannose type, and the acidic oligosaccharides were of the complex type with three different types of outer chains in various combinations: +-NeuAcalpha2 to 6Galbeta1 to 4GlcNAc; Galbeta1 to 3Galbeta1 to 4GlcNAc; and Galbeta1 to 4GlcNAcbeta1 to 3Galbeta1 to 4GlcNAc. An interesting finding was that two sugar chains, NeuAcalpha2 to 6Galbeta1 to 4GlcNAcbeta1 to 2Manalpha1 to 3(Galbeta 1 to 4GlcNAcbeta1 to 2Manalpha1 to 6)Manbeta1 to 4GlcNAcbeta1 to 4(+-Fucalpha1 to 6)GlcNAcOT (in which the subscript OT indicates a NaB3H4-reduced oligosaccharide), were found only in surface carbohydrates of D5A1. (Author abstract) (24 Refs)

3. GLYCOSYL TRANSFERASES: BIOLOGY AND CLINICAL SIGNIFICANCE.

Huckel C, Brock J
Institut fur Physiologische Chemie des Bereichs Medizin, Wilhelm-Pieck-Universitat Rostock, Leninallee 70, DDR-2500 Rostock, E. Germany
Wiss Z Univ Rostock (Math); 30(3):41-44 1981

Studies of glycosyl transferases (GT), their regulation, and relationships to tumor growth are reviewed. GT are located within the Golgi apparatus, endoplasmic reticulum, and evidently on the surface membranes of cells. GT apparently play a role in recognition of cells by the immune system. Variations in expression of GT may be of diagnostic and/or prognostic value in neoplastic diseases. Whether tumor cells have completely distinct forms of GT has not been conclusively determined. (41 Refs) (In GER)

4. SULFATED GLYCOSAMINOGLYCAN AND COLLAGEN PATTERNS IN PARIETAL YOLK SAC CARCINOMA (PYSC).

De Fatima Bonaldo M, Cassaro-Strunz CM, Machado-Santelli G
Fundacao 'Centro de Pesquisa de Oncologia', P.B. 11.490, Sao Paulo, Brazil
Cell Differ; 11(2):99-106 1982

Collagen and sulfated glycosaminoglycans (GAG) were analyzed in mouse parietal yolk sac carcinoma (PYSC) in vivo and in vitro, to correlate the substances with basement membrane synthesis. The collagen-like polypeptide patterns obtained from the incubation medium material of PYSC are presented. Two polypeptide components were found to migrate as type I collagens, and the analysis of the alpha1/alpha2 ratios ranged from approx 2-3. The change observed in the pattern of GAG sulfate in the solid tumor, inverting the proportion of heparan sulfate and chondroitin sulfate B, could be a consequence of the cellular differentiation that was observed at a growth rate which occurred during development of the tumor. (32 Refs)

5. HUMAN OSTEOSARCOMA DERIVED CLONAL VARIANTS: STABLE DIFFERENCES IN CELL SURFACE COMPOSITION.

De Martelaere D, Cassiman JJ, Van Leuven F, Verlinden J, Marynen P, Casteleyn J, Van den Berghe H
(c/o Cassiman) Div. Human Genetics, Dept. Human Biology, Univ. Leuven, Minderbroedersstraat 12, B-3000 Leuven, Belgium
Cell Biol Int Rep; 6(5):489-494 1982

Expression of a2Macroglobulin receptor, aminopeptidase M, transglutaminase, and fibronectin was measured by immunochemical and enzymatic techniques on five clonal variants of human osteosarcoma cell line MG-63. Major quantitative interclonal differences were observed in expression of the surface components, including reduced to absent expression of one or more components in some of them. (13 Refs)

6. CELL SURFACE PROPERTIES INFLUENCING TARGET CELL SENSITIVITY FOR NK LYSIS.

Kiessling R, Gronberg A, Golstein P, Mayer M, Henney C, Herberman R, Perlmann P, Lachman P
Dept. Tumor Biology, Karolniska Institutet, S 104 01 Stockholm, Sweden
Adv Exp Med Biol; 146(82):367-374 1982

Different cell surface properties which have been suggested to be of importance in conferring natural killer (NK) susceptibility to a target cell are summarized focusing on recent studies. Examples of 'non-specific' and 'specific' cell membrane properties with a reported influence on rendering a target cell susceptible to NK lysis are presented. Results suggested that asialo GM2 alone may not be the target structure for NK cells and the concentrations of higher ganglioside homologous with migration of GM1, GD1a, GD1b, and GT correlated positively with sensitivity of target cells to NK cell mediated lysis. (30 Refs)

7. METASTATIC PROPERTIES AND CELL SURFACE BIOCHEMISTRY OF CLONES ISOLATED FROM AN HSV-2 TRANSFORMED HAMSTER CELL LINE (MEETING ABSTRACT)

Walker JR, Rees RC, Potter CW

Clinical Research Laboratory, Weston Park Hospital, Sheffield, England

Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

After resection of subcutaneous tumor derived from the inoculation of the HSV-2-333-2-26 cell line into Syrian golden hamsters, a small proportion of animals developed metastatic lesions at secondary sites. Selection of variants by subcutaneous re-implantation of lung foci has produced two sublines with a markedly increased metastatic potential when compared with the parent line. In addition, numerous differences in cell surface glycoproteins labelled by the galactose oxidase - tritiated sodium borohydride technique have been reported. The study of these cell lines has been extended by the repeated subcutaneous transplantation of lung and kidney foci in order to produce lines of different organ propensity. The parent, poorly metastatic cell line has been cloned in vitro and a range of clones of different malignancy has been obtained. Preliminary studies have suggested that some cell surface glycoprotein components also differ between the cell lines. (1 Ref)

8. TWO-DIMENSIONAL GEL ELECTROPHORESIS OF MEMBRANE PROTEINS FROM THE R3327 PROSTATE ADENOCARCINOMA.

Kozlovskis PL, Claflin AJ, Fletcher MA, McKinney EC, Rubin RW

Dept. Medicine (D-39), Univ. Miami Sch. Medicine, P.O. Box 016960, Miami, FL, 33101

Cancer Res; 42(7):2748-2756 1982

The Dunning rat prostate adenocarcinoma (R3327) is a reliable model that shares many similarities with the human tumor. Two sublines of the tumor, G and H, represent opposite extremes in histology and growth rate. Purified membrane fractions from G and H solid tumors were isolated by sucrose gradient. Tumor and normal prostate membrane proteins were labeled with 125I, incubated with G or H antisera, and precipitated by absorption of antibody-antigen complexes to staphylococcal Protein A. Proteins were resolubilized and electrophoresed on two-dimensional gels, and the gels were autoradiographed. A total of eight labeled proteins were precipitated from the G and H tumors in the presence of G antisera. Of these, seven were homologous. One high-mol-wt protein (Protein b) present on the G tumor was absent from the H tumor. The H tumor contained high-mol-wt protein (i) that was not found on the G tumor or on normal prostate. Normal prostate revealed a pattern similar to the G tumor except that Protein b appeared to be quantitatively reduced. Precipitation in the presence of H antisera showed similar patterns except that Protein b was not detected in the G tumor and was greatly reduced in the normal prostate. Therefore, despite variable growth characteristics, there were few changes in membrane proteins between the solid tumors and between the tumors and normal prostate. Iodination of surface proteins of cultured cells from normal prostate and the G and H sublines also showed a high degree of homology. No consistent differences between cultured cell lines were noted. (Author abstract) (27 Refs)

9. ISOLATION AND CHARACTERIZATION OF PLASMA MEMBRANES FROM KREBS II ASCITE CELLS USING PERCOLL GRADIENT.

Record M, Bes JC, Chap H, Douste-Blazy L

Inserm Unite 101, Biochimie des Lipides, Hopital Purpan, 31059 Toulouse, France

Biochim Biophys Acta; 688(1):57-65 1982

Plasma membranes were isolated from Krebs II ascites cells grown in the mouse. Cells were disrupted by nitrogen cavitation in an isotonic alkaline buffer containing magnesium and ATP. Isolation was performed in an alkaline-buffered self-generating gradient of Percoll with an angular rotor. At each step of the preparation, the pH appeared as the critical aspect of our procedure. External membrane markers were concanavalin A and 5'-nucleotidase (EC 3.1.3.5). They reached a relative specific activity of 10, whereas this value was only of 0.7 for the endoplasmic reticulum marker, NADH dehydrogenase (EC 1.6.99.3). Plasma membrane from 4 ml packed cells were isolated within 1 hr after homogenization with good yield: 50% and 67% of total [3H]concanavalin A and 5'-nucleotidase, respectively, were recovered in the two plasma membrane fractions. Electron microscopy examination showed the presence of vesicles of different sizes devoid of other structural contaminants. Using the specific binding of concanavalin A to the external cell membrane, it was calculated that about 50% of the total cell phospholipid and 10% protein are located in the plasma membrane. Their sphingomyelin content is much higher than in the whole cell, in contrast to phosphatidylinositol, known as a more specific endoplasmic reticulum phospholipid. (Author abstract) (52 Refs)

10. THE ANALYSIS OF INTERNALLY RADIOLABELLED MEMBRANE AND SUPERNATANT PROTEINS PRODUCED BY T LYMPHOBLASTOID CELL LINES.

DeMartino JL

University of Massachusetts, Boston, MA

Diss Abstr Int [B]; 42(12,pt1):4738-B 1982

Proteins from the membranes and culture media of nine, human T lymphoblastoid cell lines and of the myeloid line K 562 were analyzed after [35S] methionine metabolic labelling by electrophoresis in SDS (sodium dodecyl sulfate) polyacrylamide gradient gels. A monotonous consistency was present among these cell lines in their synthesis of membrane proteins. In contrast, the synthesis of culture supernatant proteins was found to differ substantially among the cell lines. Proteins of 35,000 and 36,000 daltons were most prominently synthesized by 1301. Proteins of 50,000 daltons were abundantly synthesized by SKW3 and 8402. Proteins of 65,000 daltons were best synthesized by Jurkat, 1301 and CEM. Synthesis of a CEM supernatant protein of 35,000 daltons (p35), which is scarcely expressed on CEM membranes, increased 16-fold when cells were grown in crowded cultures. Crowded cultures of 1301 but not KE 37 exhibited increased synthesis of p35, while Raji, the only B cell line tested, did not show any synthesis of p35. The view was proposed that cultured T lymphoblastoid malignancies do not represent static vignettes of stages of differentiation of normal T lymphocytes. (no Refs)

11. MODIFYING EFFECT OF VINBLASTINE ON SUPEROXIDE ANION PRODUCTION BY MEMBRANE PERTURBING AGENTS IN POLYMORPHONUCLEAR LEUKOCYTES.

Okamura N, Ishibashi S

Dept. Physiological Chemistry, Hiroshima Univ. Sch. Medicine, Minami-ku, Hiroshima, Hiroshima 734, Japan

J Biochem (Tokyo); 91(5):1725-1730 1982

Superoxide anion production in peritoneal polymorphonuclear leukocytes obtained from guinea pigs was stimulated by in vitro treatment with membrane-perturbing agents, such as cytochalasin D, concanavalin A, phorbol myristate acetate, myristate, digitonin, and NaF. Vinblastine modified these stimulating effects on the superoxide anion production, but its modifying effect was not uniform. The effect of cytochalasin D was stimulated by vinblastine at the concentration of 10(-5)-10(-7) M, whereas it was inhibited at the concentration of 10(-4) M. At 10(-4)-10(-5) M, vinblastine was inhibitory to the effect of concanavalin A, and lower concentrations had no significant effect. Stimulation of the superoxide anion production by phorbol myristate acetate and myristate was further enhanced by vinblastine at any concentration in the range of 10(-4)-10(-8) M with peaks at 10(-6) and 10(-5) M, respectively. Vinblastine had little effect on the stimulation of the superoxide anion production by digitonin and NaF throughout the concentration range examined. The mechanism of the interaction of these membrane-perturbing stimulants and vinblastine is discussed. (Author abstract) (24 Refs)

12. HUMAN LYMPHOMA-LYMPHOMA HYBRIDS AND LYMPHOMA-LEUKEMIA HYBRIDS. I. ISOLATION, CHARACTERIZATION, CELL SURFACE MARKERS, AND B-CELL MARKERS.

Zeuthen J, Klein G, Ber R, Masucci G, Bisballe S, Povey S, Terasaki P, Ralph P

Inst. Human Genetics, Univ. Aarhus, The Bartholin Building, DK-8000 Aarhus C, Denmark

JNCI; 68(2):179-195 1982

Four new somatic cell hybrids were obtained by fusion of various Burkitt's lymphoma (BL)-derived cell lines that had different selective markers: Raji-P3HR-1, Daudi-Raji, and a P3HR-1-P3HR-1 'autohybrid' derived from two P3HR-1 sublines. In addition, a hybrid was was obtained between the Daudi (BL) line and the human leukemia cell line K562. The hybrids were extensively characterized by means of chromosome, isozyme, and HLA surface markers. The phenotypic differences between the parent cell lines allowed some conclusions with respect to the expression of latent Epstein-Barr virus (EBV) genomes, C3 and EBV receptors, and of immunoglobulin and beta 2-microglobulin-HLA expression as well as the influence of the leukemia cell (K562) genome on B-cell properties in the Daudi-K562 hybrid. B-cell and differentiated markers of these hybrids were characterized. High-level expression dominated for the markers C3 and EBV receptors, which showed a good correlation coefficient of 0.84, as was true for Fc receptors and surface immunoglobulin. The Daudi-K562 hybrid showed loss of all B-cell markers but retention of the leukemia cell markers (eg, hemoglobin synthesis). (Author abstract) (59 Refs)

13. STUDY OF HUMAN MELANOMA CELL SURFACE ANTIGENS USING MONOCLONAL ANTIBODIES.

Yeh MY

University of Washington, Seattle, WA

Diss Abstr Int [B]; 42(12,pt1):4742-B 1982

BALB/c mice were immunized with cultivated cells from one of two melanomas, M1804 or SK-MEL 28. Spleen cells from hyperimmunized mice were fused with mouse NS-1 myeloma cells. Hybrid cells were grown in HAT (hypoxanthine, aminopterin, thymidine) selective medium and screened for production of antibodies that bound to melanoma cells but not to autologous skin fibroblasts. Five hybrids were selected for extensive studies. Three hybridomas, 3.1, 3.2, and 3.3 form monoclonal antibodies to the same antigenic determinant, called 3.1 which is strongly expressed by the immunizing melanoma, M1804. Binding assays performed with a large panel of cultured human cells have demonstrated that antigen 3.1 is expressed strongly by a small subgroup of cultured melanomas, less strongly by another group of melanomas or not at all by a third group of melanomas. It is not detected on cultures from most non-melanoma tumors, normal skin fibroblast lines, or on B- or T-cells lines. Hybridomas 4.1 and 4.2 were generated against melanoma line SK-MEL 28. Hybridoma 4.1 produces an IgG antibody which identifies a cell surface protein with a molecular weight of 97,000. This protein, designated p97, is strongly expressed by both cultured cells and biopsy material from melanomas and certain other tumors. Cells from various normal tissues express only low amounts of p97. Hybridoma 4.2 secretes an IgM antibody which recognizes an antigen shard by about 95% of melanoma lines and 25% of cultured non-melanoma tumors tested. (no Refs)

14. A HUMAN THY-1-LIKE ANTIGEN EXPRESSED ON CULTURED LEUKEMIC T-CELLS.

Saji F, Tanigaki N

(c/o Tanigaki) Dept. Molecular Immunology, Roswell Park Memorial Inst., Buffalo, NY, 14263

Immunogenetics; 15(6):551-563 1982

A human cell membrane antigen that is highly T-cell specific among a number of leukocyte cell lines was isolated from cells of a human T-cell leukemia cell line, SKW-3. In addition to the high specificity to T-cell-type cell lines, the isolated antigen showed the following characteristics: (1) It is an acidic glycoprotein of approximately 30,000 daltons that has a charge heterogeneity and probably a disulfide bond(s); (2) Its antigenicity is stable when treated with heat, acid, and various protein denaturants; (3) It is widely distributed in lymphoid and nonlymphoid tissues but is most predominant in brain. These features are similar, if not identical, to those reported for the Thy-1 antigen of mouse or rat and have raised the possibility that this cell membrane antigen may correspond to human 'lymphocyte' Thy-1 antigen, the counterpart of human 'brain' Thy-1 antigen. (Author abstract) (28 Refs)

MORPHOLOGICAL STUDIES

15. ENDOCYTOSIS OF NERVE GROWTH FACTOR BY PC12 CELLS STUDIED BY QUANTITATIVE ULTRASTRUCTURAL AUTORADIOGRAPHY.

Hogue-Angeletti R, Stieber A, Gonatas NK
Div. Neuropathology, Dept. Pathology, Univ. Pennsylvania Sch. Medicine, Philadelphia, PA, 19104
Brain Res; 24(1):145-156 1982

Ultrastructural quantitative autoradiography was used to investigate endocytosis of 125I-nerve growth factor (NGF) by cultured rat pheochromocytoma cell line PC12. Cells not previously exposed to NGF were incubated at 37 C with 125I-NGF for 2, 6, or 26 hr. Outgrowth of neurites has not been seen under such conditions. At 2 hr, the plasma membrane was heavily labeled; at 6 and 26 hr, less but significant amounts of labeling was observed. Endocytosis of 125I-NGF was observed at all time periods. 125I-NGF was initially associated with the plasma membrane and underwent endocytosis very slowly. Distinct endocytosis had occurred after incubation for 6 hr, but grains were still present at the plasma membrane and the nuclei were not labeled. At 26 hr, intracytoplasmic grains appeared in clusters over dense round bodies, representing secondary lysosomes. The number of grains over the plasma membrane, the Golgi apparatus, and the smooth endoplasmic reticulum did not change significantly between 6 and 26 hr. The pathway of endocytosis of 125I-NGF apparently included vesicles of the smooth endoplasmic reticulum, but not the Golgi apparatus. These findings are consistent with the hypothesis that NGF constantly occupies sites on or adjacent to the plasma membrane, and that it slowly undergoes endocytosis into lysosomes. (33 Refs)

16. DECREASED INCIDENCE OF GAP JUNCTIONS BETWEEN CHINESE HAMSTER V-79 CELLS UPON EXPOSURE TO THE TUMOR PROMOTER 12-O-TETRADECANOYL PHORBOL-13-ACETATE.

Yancey SB, Edens JE, Trosko JE, Chang CC, Revel JP
Div. Biology 156-29, California Inst. Technology, Pasadena, CA, 91125
Exp Cell Res; 139(2):329-340 1982

Previous studies have shown that metabolic cooperation between Chinese hamster V-79 cells is inhibited by the tumor-promoting phorbol esters, but not by those phorbol esters inactive in tumor promotion. Metabolic cooperation is believed to be mediated by gap junctions. Therefore, in the present study we have used freeze-fracture and quantitative morphological techniques to examine the effect of the most potent tumor-promoting phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate (TPA) and of the inactive analog, 4alpha-phorbol-12, 13-didecanoate (4alpha-PDD) on the frequency of gap-junctional contacts between V-79 cells. In both control and in 4alpha-PDD treated cultures the junctions were frequent, though rather small in size. In contrast, in the TPA-treated cultures, gap junctions were few, and the area of membrane occupied by gap junctions was reduced more than 20-fold from that found for controls. These results suggest that the TPA-induced inhibition of metabolic cooperation between V-79 cells is a consequence of a decrease in the number of gap junctions. (Author abstract) (61 Refs)

17. ULTRASTRUCTURE OF HUMAN NATURAL KILLER CELLS: NATURE OF THE CYTOLYTIC CONTACTS IN RELATION TO CELLULAR SECRETION.

Carpen O, Virtanen I, Saksela E
(c/o Saksela) Dept. Pathology, Univ. Helsinki, Haartmaninkatu 3, 00290 Helsinki 29, Finland
J Immunol; 128(6):2691-2697 1982

The ultrastructure of human NK cells, NK/target cell conjugates and the effect of monensin, a secretion inhibitor, were studied. Human peripheral blood lymphocytes, highly enriched (75 to 85%) in large granular lymphocytes (LGL), which are known to be the mediators of human NK activity, were used as effectors, and K562 cells as targets. LGL-type of cells were characterized in electron microscopy by a low nucleocytoplasmic ratio, indented nucleus, several large mitochondria, prominent Golgi apparatus, and cytoplasmic osmiophilic granules bound by a unit membrane. Their surface was of intermediate villous type. Two types of effector/target contacts were seen: either effector cell protrusions were pushed deep into pouches and lacunae of the target cell surface, or a wide area of intimate cell-to-cell contact was formed. The contact formation was followed by polarization of the effector cell Golgi apparatus towards the contact area. Monensin, a carboxylic ionophore, which interrupts the vesicular traffic of Golgi-derived vacuoles to the cell surface, caused an accumulation of cytoplasmic vesicles in the LGL but had no effect on the effector/target contact formation. Monensin inhibited the NK lysis apparently via cessation of secretion by the LGL. It did not affect the viability of the effector cells. The lytic steps of human NK cells thus involve binding to the target cell, polarization of cytoplasmic organelles towards the target, and apparently a precisely directed exocytosis of secretory material. (Author abstract) (32 Refs)

18. AN EM STUDY OF ALKALINE PHOSPHATASE IN A TRANSPLANTABLE OSTEOGENIC SARCOMA OF THE RAT (MEETING ABSTRACT)

Ingleton PM, Gaitens PV, Coulton LA, Russell RG
Department of Zoology, The University of Sheffield, Sheffield S10 2TN, England
Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

This report details ultrastructural investigations, using Gomori's technique, into the site of alkaline phosphatase (AP) activity in the tumor, during growth after implantation. After only 15 days growth no distinct areas could be discerned in the tumor nodule, but AP activity was seen in cells throughout, particularly in golgi membranes, as discrete punctate areas on plasma membranes and as tiny spots on matrix vesicle membranes. Active cells of areas, designated A - D, from tumors growing for 22 to 35 days had membrane associated AP activity, even when dividing. Area C contained a high proportion of necrotic tissue and no AP activity could be detected in the cell debris. Organized collagen developed intercellularly during tumor growth and AP activity was detected in discrete bodies associated with collagen. Crystalline calcium spicules also developed apparently from AP containing bodies, whether on cell membranes or collagen bundles, notably in area D after 35 days growth. The close association of AP activity with tumor cells during early stages of tumor growth, and later appearance intercellularly and in venules, correlates with previous in vivo observations that serum AP concentrations only rise significantly after the tumor has been growing for at least 11 days. (3 Refs)

19. IMPORTANCE OF CYTOPLASM/CELL MEMBRANE DAMAGE INDUCED BY ADRIAMYCIN (MEETING ABSTRACT)

Walling JM, Ord MJ

Department of Biology, University of Southampton, Hants S09 3TU, England

Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

In the present study adriamycin was found to exhibit a biphasic pattern of toxicity towards A proteus and CHO (Chinese hamster ovary) cells. Cells were either killed very rapidly as a result of complete loss of membrane integrity and consequent organelle destruction, or more slowly over a period of up to eight days following removal of the treatment solution as assayed by a single cell cloning. EM (electron microscopy) studies on amebae showed aberrant mitochondrial profiles with a reduction in the number of internally located cristae and a widening of the peripherally located cristae. Very aberrant mitochondria were eventually sequested in autophagocytic vacuoles. The typical anthracycline induced nucleolar fragmentation was often seen in later stages of pathogenesis. However, this was never observed without concomitant cytoplasmic damage. The results of an initial series of nuclear transfers between treated and control amebae suggest that treated cytoplasms have a far poorer ability to survive than do treated nuclei. Taken together the results with both amebae and CHO cells suggest that damage to the cytoplasm/cell membrane may contribute considerably to adriamycin induced cell death. (2 Refs)

20. ULTRASTRUCTURAL AND MORPHOGENETIC RESPONSES TO VARYING CALCIUM CONCENTRATIONS BY HUMAN CERVICAL CARCINOMA CELLS (MEETING ABSTRACT).

Auersperg N, Swiergenga SH, Feng S

Anatomy Department, University of British Columbia, Vancouver V6T 1W5, British Columbia, Canada

In Vitro; 18(3,part2):305 1982

Cells of the cervical carinoma line C-4I have been propagated for over 100 passages in Waymouth' medium 752/1 and 10% fetal bovine serum. The cells form cohesive multilayered colonies with tonofilament bundles, desmosomes and an intercellular organization resembling stratified squamous epithelium. When crowded C-4I cultures were exposed to Williams medium E with Ca++ reduced to 0.02 mM, most cells detached and only a monolayer remained on the culture dishes. It maintained at high densities, these cells replicated in 0.02 mM Ca++ and could be selected to grow either in suspension or as monolayers. The monolayered C-4I cells, like epidermal keratinocytes grown in media with low Ca++, were rounded or flat, lacked desmosomes and had centrally displaced tonofilaments. Upon return of C-4I cells to media with standard Ca++ concentrations the morphologic charactistics of stratified squamous epithelium were rapidly restored. Squamous cells with cytoplasmic granules and a very low nucleocytoplasmic ratio appeared in greater numbers in 1.8 mM than in 0.82 mM Ca++. The responses of C-4I cells to varying extracellular Ca++ levels resemble those of normal keratinocytes and some epidermal carcinoma cells which also differentiate in high, but not in low extracellular Ca++. (no Refs)

MEMBRANE RECEPTOR/ BINDING SITES

21. MONOCLONAL ANTIBODIES TO THE P21 PRODUCTS OF THE TRANSFORMING GENE OF HARVEY MURINE SARCOMA VIRUS AND OF THE CELLULAR RAS GENE FAMILY.

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Lab. Tumor Virus Genetics, NCI, Bethesda, MD, 20205

J Virol; 43(1):294-304 1982

We have isolated eight rat lymphocyte-myeloma hybrid cell line producing monoclonal antibodies that react with the 21,000-dalton transforming protein (p21) encoded by the v-ras gene of Harvey murine sarcoma virus (Ha-MuSV). These antibodies specifically immunoprecipitate both phosphorylated and non-phosphorylated forms of p21 from lysates of cells transformed by Ha-MuSV. All eight react with the products of closely related ras genes expressed in cells transformed by two additional sarcoma viruses (rat sarcoma virus and BALB sarcoma virus) or by a cellular Harvey-ras gene placed under the control of a viral promoter. Three of the antibodies also react strongly with the p21 encoded by the v-ras gene of Kirsten MuSV. These same three antibodies immunoprecipitate the predominant p21 species synthesized normally in a variety of rodent cell lines, including the p21 produced at high levels in 416B murine hemopoietic cells. This suggests that an endogenous gene closely related to Kirsten-ras is expressed in these cells. The monoclonal antibodies have been used to confirm two properties associated with p21: localization at the inner surface of the membrane of Ha-MuSV-transformed cells, assayed by immunofluorescence microscopy, and binding of guanine nucleotides. (Author abstract) (42 Refs)

22. THE INCREASE IN HORMONE-STIMULATED ADENYLATE CYCLASE ACTIVITY FOLLOWING ROUS SARCOMA VIRUS TRANSFORMATION.

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J Cell Physiol; 111(3):295-302 1982

Ability of prostaglandin E1 (PGE1) and isoproterenol (ISP) to elevate the intracellular concentration of cyclic AMP (cAMP) was studied in uninfected cells, cells infected and transformed by Rous sarcoma virus (RSV) and Rous associated virus (RAV). Uninfected chicken embryo cells (CEC), cells infected but not transformed by RAV, and RSV-transformed cells in culture immediately synthesized increased amounts of cAMP upon exposure to 10 uM ISP, 10 uM PGE1, or prostaglandin E2. RSV-transformed cells were consistently more responsive to maximal stimulatory concentrations of ISP than uninfected and RAV-infected cells. RSV-transformation was observed to alter the adenylate cyclase activity measured by CEC membrane preparations. The time courses for the hormone stimulation of intact RSV-transformed and RAV-infected cells suggested that the untransformed cells were rapidly desensitized during continued beta-adrenergic receptor stimulation, while the transformed cell cultures continued to respond to ISP. Results indicated that transformed cells possess a greater number of beta-adrenergic receptors, and are more slowly desensitized by stimulatory concentrations of ISP. Membranes prepared from transformed cells specifically bound greater than 5x the quantity of radiolabeled (-)3H-dihydroalprenolol and 125I-iodocyanopindolol as compared with the untransformed cell membranes suggesting that the major difference in

the untransformed and transformed cells is in the concentration of membrane beta-adrenergic receptors and the inability of RSV-transformed cells to self-limit their response to specific external stimuli. (43 Refs)

23. EFFECTS OF AMINE SUBSTANCES ON SUSCEPTIBILITY OF CELLS TO AGGLUTINATION BY CONCANAVALIN A AND ON PARACRYSTAL FORMATION BY VINBLASTINE IN UNTRANSFORMED 3T3 CELLS.

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Dept. Anesthesiology, Tohoku Univ. Sch. Dentistry, Sendai 980, Japan
Tohoku J Exp Med; 137(1):91-99 1982

A secondary amine (ketamine), tertiary amines (dibucaine, lidocaine, marcaine, propanidid, diazepam and chlorpromazine) and a quaternary amine (tetraethylammonium bromide) were found to increase susceptibility of 3T3 cells to agglutination by Con A, as was the mixture of colchicine and cytochalasin B. This increased susceptibility of cells to agglutination by Con A with amines was inhibited by addition of 1 mM or 10 mM divalent calcium. Ketamine, propanidid, chlorpromazine and tetraethylammonium bromide greatly increased the number of cells with Con A receptors stained in a patch form. Con A receptors, while incubated at 0 C with low concentrations of Con A and amines, formed patches on the cell surface suggesting that amines redistribute Con A receptors from a random pattern into patches without involving metabolic components. It was also shown that divalent calcium ionophores and amines decreased paracrystal formation by vinblastine in the cytoplasm. The decrease in the number of paracrystal forming cells after treatment with amines may be due to inhibition of profilaments formation, or impaired microtubule formation caused by increased intracellular divalent calcium released from acidic phospholipids to which the amines bind. (32 Refs)

24. INHIBITION OF EHRLICH ASCITES TUMOR CELL GROWTH BY GRIFFONIA SIMPLICIFOLIA I LECTIN IN VIVO.

Eckhardt AE, Malone BN, Goldstein IJ
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Cancer Res; 42(8):2977-2979 1982

Griffonia (Bandeiraea) simplicifolia I (G I) seeds contain a family of alpha-D-galactopyranosyl-binding isolectins which strongly agglutinate Ehrlich ascites tumor cells due to the presence of this determinant sugar on their cell surface glycoproteins. Administration of GS I lectin (100 ug/day or 300 ug on alternate days, ip) inhibits the growth of Ehrlich tumor cells in vivo. Mice given injections of only Ehrlich cells lived an average of 36 +-13 (SE) days with no mouse surviving longer than 53 days. Mice given injections of Ehrlich cells and 100 ug GS I lectin daily or 300 ug GS I lectin on alternate days showed 75 and 62% survival, respectively, after 60 days. Lectin-dependent, macrophage-mediated tumor cell lysis may be the mechanism whereby the GS I lectin exerts its cytotoxic effect. (Author abstract) (14 Refs)

25. MITOGENIC LECTIN RECEPTORS OF NERVOUS SYSTEM TUMORS. STUDY OF GLIOMAS, NEURAL CREST TUMORS, AND MENINGIOMAS IN VITRO USING PHYTOHEMAGGLUTININ AND CONCANAVALIN A.

Liwnicz BH
Dept. Pathology and Lab. Medicine, Univ. Cincinnati, Coll. Medicine, 231 Bethesda Ave., Cincinnati, OH, 45267
J Neuropathol Exp Neurol; 41(3):281-297 1982

The effects of mitogenic lectins phytohemagglutinin (PHA), and concanavalin A (Con A) on the growth rate of cells derived from glial tumors (astrocytoma, ependymoma, glioblastoma, medulloblastoma, and C6 rat glioma), neural crest tumors (neuroblastoma and schwannoma), and meningiomas were studied. The cell lines were of human and animal origin. The specificity of lectin binding to mitogenic receptors was evaluated using complementary monosaccharides. In all glial- and some neural-crest tumor-derived cell lines, there was a lectin concentration-dependent and cell density-dependent, biphasic growth rate response with stimulation at low and inhibition at high lectin concentrations. This response did not depend on the type of glial tumor, species of origin, or passage level in vitro. Although, in meningioma-derived cell lines, lectins did not induce a growth rate response, they caused morphological changes ('whorling'). Lectin stimulation in glial tumor-derived cell lines resembles that occurring in peripheral blood lymphocytes. Lectin-induced mitogenesis may lay the groundwork for the establishment of a model of glial cell proliferation, and that permits the evaluation of cell surface effects, intracellular mechanisms, and epigenetic factors in studies of tumors, neural development, and neuroimmunology. (Author abstract) (28 Refs)

26. FLUORESCEINATED LECTINS AS PROBE FOR CELL SURFACE CHANGES ASSOCIATED WITH LYMPHOCYTE TRANSFORMATION.

Strauchen JA
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Am J Hematol; 12(3):227-232 1982

Lectins are plant-derived proteins with specific affinities for simple or complex sugars and are potentially useful tools for investigation of the carbohydrate structure of the cell surface. A panel of fluoresceinated lectins was used to study the cell surface changes associated with lymphocyte transformation. The major determinants detected on peripheral blood lymphocytes were alphaD-mannose,beta-D-galactose and N-acetyl-glucosamine. Phytohemagglutinin transformation was associated with increased binding of lectins specific for N-acetyl-galactosamine. MOLT-4, a T-lymphoblast cell line in continuous culture, was associated with increased binding of lectins specific for N-acetyl-galactosamine and L-fucose. Application of fluoresceinated lectins may be a useful tool in the classification of lymphoid cells. (Author abstract) (10 Refs)

27. PURIFICATION AND CHARACTERIZATION OF A MAJOR CELL SURFACE GLYCOPROTEIN IN ZAJDELA ASCITES HEPATOMA CELLS WHICH DISPLAYS A POTENT CONCANAVALIN A RECEPTOR ACTIVITY.

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J Cell Biochem; 18(2):245-260 1982

The isolation and characterization of a major cell glycoprotein from Zajdela hepatoma ascites (ZHA) cells, which be-

haves as a Concanavalin A receptor, was studied to determine which role the cell surface proteins might play in the tumor growth in the system. Effects of trypsin treatment on the amount of radioactivity and protein released from the hepatoma cells are presented. Cell viability after the treatment was greater than 95% and treated cells after 8 hr culture regained the same proliferation capacity as intact cells. Electrophoresis of trypsin digest revealed essentially a single radioactive band strongly stained by periodate-Schiff reagent and a few weakly stained bands. Glycoprotein (GP) II2 appeared homogenous and could be a major constituent released from ZHA cell surface. Carbohydrates represented 32% of the GP II2 molecule, indicating that mol wt of the carbohydrate-free polypeptide could be 72,000 daltons. An indication of GP II2 location was obtained from the cell viability following controlled proteolysis and by specific labeling of sialyl residues with a reagent that theoretically does not enter into the cells. In addition, the metabolic labeling of GP II2 with D-(3H)-glucosamine showed that the GP originated from the cell itself and was not an ascites ligand GP firmly bound to the plasma membrane and hence labeled by the surface labeling procedure. The binding of Concanavalin A on hepatoma cells in the presence of methyl alpha-D-mannoside (0.2 M) or GP II2 (0.01 milliM) was inhibited at 85% and 72%, respectively. Thus, GP II2 proved to be an extremely potent inhibitor of both Concanavalin A binding to the cell surface and its effects on cell proliferation. (55 Refs)

28. BINDING OF WHEAT GERM LECTIN TO RAT SARCOMAS OF DIFFERING METASTATIC CAPACITY (MEETING ABSTRACT)

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Department of Oncology, University of Glasgow, Glasgow, Scotland

Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

The reaction of Wheat Germ Lectin with the tumor cell surface (as expressed by amount bound, agglutination, resistance to toxic effects) has been reported to correlate with metastatic potential in a number of parisophanous tumor cell lines (ie tumors derived from a parental line). We are currently examining a range of rat sarcomas of independent origin to see if there is any correlation between binding of Wheat Germ Lectin and metastatic capacity. The assay used to assess binding was based on the reaction of radiolabelled lectin to tumor cells in suspension and results are expressed as a percentage of an internal standard. Inhibition studies showed that Wheat Germ Lectin bound to N-acetyl glucosamine and also N-acetyl neuraminic acid. Tumor cell suspensions were prepared either by detachment of cells grown as monolayers in vitro or by mechanical disruption of a solid tumor grown in vivo, followed by removal of dead cells and debris by Ficoll-Paque, to avoid the use of enzymes. It was found that while in vitro cultured cells from tumors of different metastatic capacity showed no differences in extent of lectin binding, differences were apparent using cells from mechanically disrupted solid tumors. (no Refs)

29. THE LOSS OF CERTAIN WGA-BINDING PROTEINS IN RELATIONSHIP TO METASTASIS AND TO THE SITE OF TUMOUR GROWTH (MEETING ABSTRACT)

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Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

Recent studies have suggested that membrane protein glycosylation is important in relationship to metastasis. We have further investigated this possibility by determining the binding of 125I-Wheat Germ Agglutinin (WGA) to electrophoretically-separated proteins in Triton X100 extracts from primary and secondary tumor cells of a metastasizing hamster lymphosarcoma. It was shown that WGA binds to a number of proteins in extracts from primary tumor growing in the subcutaneous site. However, in extracted secondary cells that were isolated from the liver and lymph nodes, the degree of WGA binding was consistently reduced. Control studies showed that the observed changes were not a result of contamination, or proteolyltic digestion of the WGA-binding proteins by host cells. The lost WGA-binding bands reappeared if secondary tumor cells, that had previously grown in the liver, were then grown in the subcutaneous site. Later studies, on tumor transplanted into a number of different primary sites, indicated that the degree of WGA-binding varied according to the particular site investigated. (2 Refs)

30. LECTIN BINDING TO THE PERIPHERAL BLOOD MONONUCLEAR CELLS OF PATIENTS WITH LYMPHOMA - CORRELATION WITH CLINICAL STAGE AND OTHER SURFACE MARKERS (MEETING ABSTRACT)

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Twenty-third Annual General Meeting of the British Association for Cancer Research, March 29-31, 1982, Edinburgh, Scotland. British Association for Cancer Research 78 pp., 1982.

The binding of various lectins to the surface membranes of different cell types has been reported previously and using Con A (concanavalin A) LCA and WGA (wheat germ agglutinin), characteristic binding patterns have been obtained for Acute Myelogenous, Acute Lymphoblastic and Chronic Lymphocytic Leukemia. The peripheral blood mononuclear cells of patients with lymphoma have been studied by flow cytometry using the method previously described. It has been found that there are particular patterns of lectin binding to PBL (peripheral blood lymphocytes) cells in different pathological types of lymphoma. Those lymphomas with a poorly differentiated cell type (Rappaport) regardless of whether nodularity exists in the tumor have a repeatable pattern. Tumors with the so-called histiocytic type of cell also have a reproducible pattern of lectin binding, with a population of cells having a increased lectin binding. Cells from patients with DWDL lymphoma resembled those found in CLL. (2 Refs)

31. ADRENERGIC RECEPTORS IN HUMAN AND EXPERIMENTAL PHEOCHROMOCYTOMA.

Snavely MD, Motulsky HJ, O'Connor DT, Ziegler MG, Insel PA
Dept. Medicine, Univ. California, San Diego, La Jolla, CA, 92093
Clin Exp Hypertens [A]; 4(4/5):829-848 1982

Work characterizing alpha-adrenergic receptors on human platelets and on rat renal cortical membranes is reviewed, and results of studies of these receptors from patients and rats with pheochromocytoma are reported. Subtype-selective radioligands were used to study alpha1 (3H-prazosin) and alpha2 (3H-yohimbine) receptors in human platelets and rat renal cortical membranes. The number of yohimbine binding sites on platelets was unaltered by 4 or 24 hr incubations with 0.1 milliM epinephrine. In vitro incubation with epinephrine at concentrations which vastly exceeded physiological and even pathological plasma concentration also did not down regulate platelet alpha2-adrenergic receptors. The number of alpha2-adrenergic receptors on platelets of five patients with pheochromocytoma exposed to chronically high levels of catecholamines, remained normal as well. An animal model of pheochromocytoma is discussed. In rats with pheochromocytoma down regulation of beta- and alpha1-adrenergic receptors was demonstrated. Experimental pheochromocytoma is a useful model system for exploring down regulation of these receptor populations. (45 Refs)

32. BLOOD GROUP ABO (H) CELL SURFACE ANTIGENS IN BLADDER TUMORS INVADING THE CHORION. PROGNOSTIC VALUE OF THEIR DETERMINATION BY IMMUNOFLUORESCENCE. RETROSPECTIVE STUDY OF ONE HUNDRED AND TWENTY-FIVE CASES.

Vacant J, Le Portz B, Augustin I, Hammou JC, Fella C, Delignieres S, Maiolini R, Masseyeff R, Abelanet R, Steg A
GRET, G.I.E. FAX, 5, rue Eugene Emmanuel, 06000 Nice, France
Sem Hop Paris; 58(21):1295-1300 1982

The authors have studied 125 Stage A bladder tumors with a follow-up period of at least 5 yr. The ABH cell surface antigens were collected by a double-layer immunofluorescence technique which has already been described. All the cross sections were read by the same pathologist who knew nothing about the clinical developments. The results were divided into 3 groups: (I) all the tumor cells had retained their surface antigens. (II) Only some cells had retained surface antigens. (II) All cells were negative. Study of the development of the three groups shows that: in Group I, all the patients (11) are alive 5 yr later, and only one has presented with a lesion evolving towards infiltration. In Group II (32 patients), only 38% of the patients are still living 5 yr later, and 65% developed towards muscular infiltration. In Group III (77 patients), 27% are alive after 5 yr and 80% have developed towards muscular infiltration. Homogeneous retention of the surface antigens would therefore seem to be a favorable factor in the prognosis, even in the case of a tumor which has already infiltrated the chorion. The significance of the loss of surface antigens is less clear-cut, but is on the whole adverse. The significance would, however, probably be enhanced by study of the mucosa distal from the lesion, and by marking the substances which determine the blood groups. (Author abstract) (27 Refs) (In FRE)

33. EFFECT OF IONIZING RADIATION OF LYMPHOCYTE MEMBRANES. PART OF A COORDINATED PROGRAMME ON CELL MEMBRANE PROBES AS BIOLOGICAL INDICATORS IN RADIATION ACCIDENTS. FINAL REPORT FOR THE PERIOD 1 APRIL 1978-31 MARCH L981.

Ojeda SF
International Atomic Energy Agency, Vienna, Austria
Effect of Ionizing Radiation of Lymphocyte Membranes. Part of a Coordinated Programme on Cell Membrane Probes as Biological Indicators in Radiation Accidents. Final Report for the Period 1 April 1978-31 March 1981. Available from National Technical Information Service, Springfield, VA, as DE81700670:, 33 pp., 1981.

A study of the effects of low doses of irradiation on membrane receptors of lymphoid cells indicated that doses as low as 10 rad induced detectable changes in the antigen receptors of cell surfaces. Lymphoid cells from mice or rabbit lymph nodes, or circulating lymphocytes from human volunteers were irradiated and studied for their ability to bind antisera against the IgG membrane receptors. The isolated lymphoid cells were x-irradiated, and tested versus non-irradiated controls. They were incubated at 37 C for different times, and IgG-positive cells stained by the direct or indirect immunofluorescence technique. The percentage of IgG-positive cells was reduced by low-dose irradiation, and proved dose- and temperature-dependent. The disappearance phenomenon depends on the microtubular structure, metabolic energy, and levels of C-AMP. Only the reappearance phase is temperature-dependent and not affected by the drugs tested. The phenomenon is dose-rate dependent, showing greater sensitivity at lower dose/rates. Experiments using anti-Fc and anti-Fab portions of the surface molecule, appear to confirm a partial internalization of the surface molecule as cause (at least in rabbit cells). Similar experiments with human cells did not show a differential effect. Human T-cells and FC receptors of Mast cells did, however, indicate that these surface molecules are also modified by irradiation. (Author abstract)

34. EVALUATION OF THE PEROXIDASE-ANTIPEROXIDASE METHOD FOR DEMONSTRATING CELL MEMBRANE B2-MICROGLOBULIN.

DiPersio LP, Weiss MA, Michael JG, Pesce AJ
Dept. Pathology, Univ. Cincinnati Medical Center, 231 Bethesda Ave., Cincinnati, OH, 45267
Am J Clin Pathol; 77(6):700-704 1982

The peroxidase-antiperoxidase (PAP) immunohistochemical technic was evaluated for its usefulness in studying B2-microglobulin (B2m) expression in the cell membrane of human tumor lines grown in athymic mice. Eleven human tumor xenograft lines expressing various amounts of B2m were used. B2m was assessed by three methods: PAP technic on formalin-fixed, paraffin-embedded tissue; indirect immunofluorescence on frozen sections; and radioimmunoassay on soluble tumor extracts. The three technics gave comparable results, and the PAP technic proved to be useful in evaluating B2m. (Author abstract) (19 Refs)

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35. ASYMMETRICAL SURFACE IGG ON MOPC-21 PLASMACYTOMA CELLS CONTAINS ONE MEMBRANE HEAVY CHAIN AND ONE SECRETORY HEAVY CHAIN.

Goding JW
Walter and Eliza Hall Inst. Medical Res., P.O. Royal Melbourne Hosp., Victoria 3050, Australia
J Immunol; 128(6):2416-2421 1982

Membrane proteins from the B lymphomas WEHI-231 and 2PK3 and from the plasmacytomas MPC-11 and MOPC-21 were radioiodinated in situ by the lactoperoxidase method and were subjected to two-dimensional (nonreduced, reduced) polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Few heavily labeled membrane proteins were composed of disulfide-bonded subunits. One such protein (mw 200,000 intact and 116,000 reduced) shared some properties with the PC-1 alloantigen, although it was not conclusively identified. A second major disulfide-bonded protein (mw 200,000 intact and 95,000 reduced) has been identified previously as the receptor for transferrin. Membrane immunoglobulins of WEHI-231 (IgM) and 2PK3 (IgG2a) had the expected subunit structure, whereas membrane immunoglobulin was not detected on MPC-11. In contrast, surface IgG1 of MOPC-21 appeared to consist almost entirely of hybrid molecules containing one membrane gamma1 chain and one secretory gamma1 chain. This hybrid IgG molecule appeared to exist in both monomeric and dimeric forms. It is concluded that (i) the synthetic and assembly mechanisms of secretory and membrane IgG1 are shared; (ii) there are no special mechanisms to prevent pairing of membrane and secretory gamma1 chains; (iii) the presence of one hydrophobic tail is sufficient for membrane insertion of gamma1 chains; and (iv) the C-terminal extension cysteine residues of membrane gamma1 chains in hybrid IgG molecules are either unpaired or may allow the formation of hybrid IgG dimers. (Author abstract) (37 Refs)

36. REDUCTION OF OPIOID BINDING IN NEUROBLASTOMA X GLIOMA CELLS GROWN IN MEDIUM CONTAINING UNSATURATED FATTY ACIDS.

Ho WK, Cox BM
Dept. Biochemistry, Chinese Univ. Hong Kong, Shatin, N.T., Hong Kong
Biochim Biophys Acta; 688(1):211-217 1982

Neuroblastoma x glioma cells NG108-15 were cultured in lipid-free medium supplemented with fatty acids of various chain length and unsaturation. Binding of 3H-labeled [DAla2]-[DLeu5]-enkephalin by membranes of cells grown in saturation fatty acids of different chain length was not significantly different from that of the control. On the other hand, a proportional decrease of binding capacity with no change in residual receptor affinity was noticed when cells were cultured in medium containing fatty acids of increasing unsaturation. This decrease was time dependent and reached a max at about 48 hr. Binding of [3H]dihydromorphine and [3H]naloxone was similarly affected. In contrast, when membranes of cells grown in normal medium were preincubated up to 3 hr with unsaturated fatty acid and tested for opioid binding, no significant reduction was observed. Examination of the fatty acid composition of phospholipid from cells grown in linolenate indicated that a significant alteration of the acyl composition has occurred. To evaluate the underlying cause of this type of inhibition, the effect of linolenic acid on cell growth and protein synthesis was examined. When cells were cultured in 100 uM of this fatty acid, both growth and protein synthesis were retarded by 28% and 19%, respectively. Since opiate receptors are proteinaceous in nature, a reduction of protein synthesis may partially account for the loss of opioid binding activity. On the other hand, an increase of membrane fluidity is known to affect a number of cellular functions, including ligand-receptor recognition. Whether this can offer a satisfactory explanation for our observations remains to be established. (Author abstract) (18 Refs)

37. EFFECTS OF DIETARY LIPIDS ON LACTOGENIC HORMONE RECEPTOR BINDING IN RAT MAMMARY TUMORS.

Cave WT, Erickson-Lucas MJ
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JNCI; 68(2):319-324 1982

Both epidemiologic studies in humans and experiments in laboratory animals have indicated that high-fat (HF) diets promote mammary tumor growth; however, the biochemical mechanisms responsible for this accelerated tumor growth are poorly understood; thus this study was designed to determine whether diet-induced alterations in the lipid composition of mammary tumor cell membranes were associated with differences in lactogenic hormone binding capacity. Mammary tumors were induced with N-methyl-N-nitrosourea in 50-day-old female inbred Buffalo rats that were maintained on either HF or low-fat (LF) diets composed of either 20% corn oil or 0.5% corn oil, respectively. The microsome-membrane fractions of these tumors were then analyzed for specific lactogenic hormone binding with the use of 125I-labeled human growth hormone. Methylated extracts of these same membrane fractions were also subjected to gas-liquid chromatography. Our results demonstrated that the mammary tumor membranes of the HF group did have a significantly greater lactogenic binding capacity than those of the LF group and that these differences in hormone binding were accompanied by significant alterations in the membrane qualitative fatty acid profiles of each group. Therefore, one way in which dietary lipids may be able to influence mammary tumor growth is by modification of the lactogenic hormone binding capacity of tumor cell membranes. (Author abstract) (27 Refs)

38. IMMUNOFLUORESCENCE STUDY OF THE DISTRIBUTION OF ABH CELL SURFACE ANTIGENS IN BLADDER TUMORS.

Vallancien G, Rouger P, Leclerc JP, Kuss R
Clinique Urologique, Hopital de la Pitie, 83, Boulevard de l'Hopital, 75634 Paris Cedex 13, France
Sem Hop Paris; 58(19):1161-1164 1982

ABH antigens are normally present at the surface of the healthy urothelium. In many cases of bladder tumors, the urothelium loses its antigens. While this loss would not seem to be associated with the grade or stage of the tumor, the persistence of the ABH antigens would seem to provide a more favorable prognosis. (Author abstract) (17 Refs) (In FRE)

39. SELECTIVE BLOCKADE OF HUMAN NATURAL KILLER CELLS BY A MONOCLONAL ANTIBODY.

Newman W
Program in Basic Immunology, Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
Proc Natl Acad Sci USA; 79(12):3858-3862 1982

A murine monoclonal antibody, 13.1, which blocks human natural killer (NK) cell-mediated lysis, has been developed. Hybridoma 13.1 was derived by fusion of NS-1 cells with spleen cells from mice immunized with an enriched population of NK cells. Supernatants of growing hybridomas were

screened for their ability to block NK cell-mediated lysis of K562 targets. Antibody 13.1 is an IgG1 with a single light chain type and it does not fix complement. The 13.1 antigen is expressed on all peripheral blood mononuclear cells, with an antigen density approx 1/30th that of HLA antigen heavy chain. Pretreatment and washing experiments revealed that inhibition of cytotoxicity occurred at the effector cell level only. Significant blocking was achieved with nanogram quantities of antibody and was not due to toxic effects on NK cells. Likewise, controls with other antibodies of the same subclass demonstrated that blocking was not a consequence of mere binding to NK cells. When a panel of 17 NK cell-susceptible targets was tested, the lysis of only 5 of these was blocked, namely K562, HL-60, KG-1, Daudi, and HEL, a human erythroleukemic cell line. The lysis of 12 human B cell and T cell line targets was not inhibited. In addition to the demonstration that the 13.1 antigen is a crucial cell surface structure involved in NK lysis, a heterogeneity of target cell recognition has been revealed that argues for the proposition that individual NK cells have multiple recognitive capabilities. (Author abstract) (28 Refs)

40. BIOCHEMISTRY OF THE ACUTE ALLERGIC REACTIONS: FOURTH INTERNATIONAL SYMPOSIUM.

Becker EL, Simon AS, Austen KF
Dept. Pathology, Univ. Connecticut Health Center, Farmington, CT, 06032
Kroc Found Ser 14: 350 pp., 1981.

Molecular and cellular biology of the acute host inflammatory responses are discussed. The biology of the mast cell, monocyte, neutrophil, eosinophil, and platelet are considered. Biochemical pathways of coupled activation/secretion, the chemistry of the granule constituents, and the chemistry and function of the secondary products such as the leukotriene constituents of the slow-reacting substance of anaphylaxis are emphasized. The acute inflammatory response are dealt with in molecular and cellular terms; structure-function relationships are indicated when possible. Other topics included are the definitions of the biochemical responses of the cell to the transmembrane signal and the definition of the multiple and possible parallel biochemical pathways that respond to membrane perturbation so as to lead to productive and integrated cell responses. Included is a chapter on structural analyses of the receptor for IgE on rat basophilic leukemia cells. (757 Refs)

41. CLOTTING FACTORS (THROMBIN, FACTOR XIII, FIBRONECTIN) AS REGULATORS OF FIBROBLAST, ENDOTHELIAL CELL AND TUMOR CELL PROLIFERATION (MEETING ABSTRACT).

Bernsmeier R, Pohl J, Bruhn HD
I Medizinische Universitats-Klinik Kiel, Kiel, W. Germany
Onkologie; 4(5):248 1981

Thrombin and factor XIII stimulate fibroblast proliferation during a simultaneous intracellular increase in cyclic guanosine monophosphate; fibronectin on the other hand inhibits the relative 3H-thymidine uptake of fibroblasts. More recent research has been able to demonstrate that vascular wall endothelial cells are stimulated in a similar fashion by thrombin and factor XIII. Comparison studies with tumor cells (HeLa, sarcoma cells) indicate that at least in some cases tumor cells are also subject to the regulations mentioned. Incubating fibroblasts with neuraminidase completely eliminates thrombin activity and factor XIII action is shifted to higher concentrations suggesting that neuraminidase either splits a fragment off a receptor or alters the receptor's conformation. (no Refs) (In GER)

42. TUMOR CELL TRANSFERRIN RECEPTOR NUMBER: AN INDICATOR OF GROWTH STATE (MEETING ABSTRACT).

Rasey JS, Larson SM, Nelson NJ
University of Washington, Seattle, WA, 98195
Program and Abstracts of the 6th Annual Meeting of the Cell Kinetics Society, March 19-21, 1982, Houston, Texas. The Cell Kinetics Society 38 pp., 1982.

Uptake of iron and other trace metals by several tumor and normal cells occurs via cell surface transferrin receptors. Iron is essential for DNA synthesis in some cells types; this is consistent with larger numbers of transferrin receptors per cell in actively proliferating compared to confluent plateau phase tissue cultures. We have determined relative number of transferrin receptors/cell and receptor affinity for transferrin in EMT-6 and RIF-1 sarcoma cells in vitro. For both cell types, receptor number begins to increase prior to the initiation of cell division, increases markedly during early exponential growth, and decreases prior to attainment of a plateau in cell number. Affinity does not change. Radiation-induced progression delay in exponentially growing cells is being used to determine if the receptor number/cell remains high in growth inhibited cultures, or, conversely, if the growth inhibition is mediated through down-regulation of receptor number. If the latter is true, assay of receptor number by binding of I-125 transferrin may be a useful kinetic technique. (no Refs)

TRANSPORT STUDIES

43. MODULATIONS OF NA+ TRANSPORT DURING THE CELL CYCLE OF NEUROBLASTOMA CELLS.

Mummery CL, Boonstra J, Van der Saag PT, De Laat SW
Hubrecht Lab., International Embryological Inst., Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
J Cell Physiol; 112(1):27-34 1982

Na+ transport properties of mouse neuroblastoma cells (clone Neuro-2A) were studied in both exponentially growing (EG) cells and during the cell cycle. In EG cells, Na+ influx followed the kinetics for a one-compartment system. The K+:Na+ ratio obtained during the cell cycle had a value between 3 and 5 during most of the cell cycle, but was significantly higher in G1 phase. The Na+ electrochemical gradient across the cell membrane was determined. The effect of amiloride was studied in various phases of the cell cycle. (60 Refs)

44. EFFECT OF EXTERNAL ATP ON THE PLASMA MEMBRANE PERMEABILITY AND (NA+ + K+)-ATPASE ACTIVITY OF MOUSE NEUROBLASTOMA CELLS.

Van Zoelen EJ, Tertoolen LG, Boonstra J, Van der Saag PT, De Laat SW
Hubrecht Lab., International Embryological Inst., Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
Biochim Biophys Acta; 720(3):223-234 1982

Addition of 3.5 mM ATP to mouse neuroblastoma Neuro-2A cells results in a selective enhancement of the plasma membrane permeability for Na+ relative to K+, as measured by cation flux measurements and electro-physiological techniques. Addition of 3.5 mM ATP to Neuro-2A cells results in a 70% stimulation of the rate of active K+-uptake by these cells, partly because of the enhanced plasma membrane permeability

for Na+. Under these conditions the pumping activity of the Neuro-2A (Na+ + K+)-ATPase is optimally stimulated with respect to its various substrate ions. External ATP significantly enhances the affinity of the Neuro-2A (Na+ + K+)-ATPase for ouabain, as measured by direct [3H]ouabain-binding studies and by inhibition studies of active K+ uptake. In the presence of 3.5 mM ATP and the absence of external K+ both techniques indicate an apparent dissociation constant for ouabain of 2.10(-6) M. Neuro-2A cells contain (3.5 +-0.7) x 10(5) ouabain-binding sites per cell, giving rise to an optimal pumping activity of (1.7 +-0.4) x 10(-20) mol K+/min per copy of (Na+ + K+)-ATPase at room temperature. (Author abstract) (48 Refs)

45. EFFECT OF FE++ IONS ON CONDUCTIVITY AND ION PERMEABILITY OF EHRLICH ASCITES CARCINOMA CELLS.

Polivoda BI, Konev VV
Res. Inst. Medical Radiology, Obninsk, USSR
Biofizika; 27(3):498-500 1982

Incubation of suspended Ehrlich ascites carcinoma cells with Fe++ ions decreased the impedance of suspension and the rate of release of ions into the culture medium. The changes in conductivity depended upon the concentration of Fe++ and the temperature. It was suggested that the mechanism of the effect of Fe++ is associated with the inhibition of ion transport by the products of lipid peroxidation. (5 Refs) (In RUS)

46. GAMMA-GLUTAMYL-TRANSPEPTIDASE ACTIVITY LEVELS IN NORMAL AND TUMOR TISSUES FROM HUMAN MAMMARY GLAND.

Puente J, Varas MA, Barrera I, Sapag-Hager M
Dept. Biochemistry, Faculty Basic and Pharmaceutical Sciences, Univ. Chile, P. O. Box 233, Santiago, Chile
IRCS Med Sci [Cancer]; 9(8):735 1981

Levels of gamma-glutamyl-transpeptidase (GGT) activity in human mammary and tumor tissues (adenocarcinoma and fibroadenoma) were determined. A significant increase in enzyme activity was found in mammary gland adenocarcinoma and fibroadenoma with respect to normal control tissue, suggesting that GGT activity is related to cellular proliferation. The difference in activity of GGT related with amino acid transport may be associated with the tumor state and its maintenance. (5 Refs)

47. MICROCYSTIS AERUGINOSA TOXIN: CELL CULTURE TOXICITY, HEMOLYSIS, AND MUTAGENICITY ASSAYS.

Grabow WO, Du Randt WC, Prozesky OW, Scott WE
Natl. Inst. Water Res., Council for Scientific Industrial Res., P.O. Box 395, Pretoria 0001, South Africa
Appl Environ Microbiol; 43(6):1425-1433 1982

Crude toxin was prepared by lyophilization and extraction of toxic Microcystis aeruginosa from four natural sources and unicellular laboratory culture. The responses of cultures of liver, lung, cervix, ovary, and kidney cell lines to these preparations did not differ significantly from one another, indicating that toxicity was not specific for liver cells. The results of trypan blue staining test showed that the toxin disrupted cell membrane permeability within a few minutes. Human, mouse, rat, sheep, and Muscovy duck erythrocytes were also lysed within a few minutes. Hemolysis was temperature dependent, and the reaction seemed to follow first-order kinetics. Escherichia coli, streptococcus faecalis, and Tetrahymena pyriformis were not significantly affected by the toxin. The toxin yielded negative results in Ames/Salmonella mutagenicity assays. Microtiter cell culture, trypan blue, and hemolysis assays for Microcystis toxin are described. The effect of the toxin on mammalian cell cultures was characterized by extensive disintegration of cells and was distinguishable from the effects of E coli enterotoxin, toxic chemicals, and pesticides. A possible reasons for the acute lethal effect of Microcystis toxin, based on cytolytic activity, is discussed. (Author abstract) (41 Refs)

48. INTERACTION OF METHOTREXATE POLY(L-LYSINE) WITH TRANSFORMED HEPATIC CELLS IN CULTURE.

Galivan J, Balinska M, Whiteley JM
Div. Lab. and Res., New York State Dept. Health, Albany, NY, 12201
Arch Biochem Biophys; 216(2):544-550 1982

Role of poly(L-lysine) as a carrier for methotrexate (MTX) was studied in H35 hepatoma cells and their transport-resistant counterparts, H35R0.3 cells. H35 andH35R0.3 cells were equally susceptible to MTX poly(L-lysine), but an 86-fold difference in the sensitivity to MTX was observed, which was reflected in the reduced uptake of MTX by the resistant subline. Both cell types accumulated much more MTX linked to poly(L-lysine) than MTX alone. MTX poly(D-lysine) was accumulated by both cell types to the same extent as the L-conjugate. MTX poly(L-lysine) completely inhibited cell growth at 200 nanoM, whereas a comparable concentration of poly(L-lysine) alone caused only a modest (less than 10%) inhibition of cell growth. MTX poly(L-lysine) exhibited extensive hydrolysis in H35 and H35R0.3 cells. Uptake of MTX by H35 cells was severely depressed in the presence of folinic acid. In contrast, inclusion of folinic acid during [3H]MTX poly(L-lysine) uptake had no effect on cellular accumulation of the unbound MTX pool demonstrating that cellular entry of the complex proceeds the liberation of MTX. Results demonstrated that MTX poly(L-lysine) enters the cells as an intact complex independent of the MTX and reduced folate coenzyme transport system. MTX poly(L-lysine) is then hydrolyzed yielding MTX by a lysosomal mechanism. The liberated MTX or its polyglutamate forms are then free to bind to the target site, dihydrofolate reductase, resulting in a blockage of folate coenzyme function. (21 Refs)

49. CHARACTERISTICS OF METHOTREXATE POLYGLUTAMATE FORMATION IN CULTURED HEPATIC CELLS.

Balinska M, Nimec Z, Galivan J
(c/o Galivan) Div. Laboratories and Res., New York State Dept. Health, Albany, NY, 12201
Arch Biochem Biophys; 216(2):466-476 1982

Formation of methotrexate (MTX) polyglutamates was studied in cultured hepatocytes and H35 hepatoma cells to elucidate the properties of the enzymic pathways which catalyze poly-gamma-glutamate synthesis and breakdown. Results demonstrated that the transport systems for the influx of MTX in hepatocytes and H35 cells are different. The major pathway for MTX entry (at concentrations greater than 1 uM) into hepatocytes appears to be related to a general anion transport pathway which is shared in some way by the bile acids and bromosulfophthalein, but not by the reduced folate coenzymes. The uptake of MTX by H35 cells is similar to other transformed cells in that it is shared by the reduced folate coenzymes, but not affected by bile acids and bromosulfophthalein. (40 Refs)

50. THE HYPOXANTHINE TRANSPORTER OF NOVKOFF RAT HEPATOMA CELLS EXHIBITS DIRECTIONAL SYMMETRY AND EQUAL MOBILITY WHEN EMPTY OR SUBSTRATE-LOADED.

Plagemann PG, Wohlhueter RM
Dept. Microbiology, Medical Sch., Univ. Minnesota, Minneapolis, MN, 55455
Biochim Biophys Acta; 688(2):505-514 1982

The kinetics of hypoxanthine transport were measured in hypoxanthine phosphoribosyltransferase-deficient Novikoff cells by rapid kinetic techniques applying both zero-trans and equilibrium exchange protocols. The data indicate operation of a simple carrier with directional symmetry and equal mobility when substrate loaded and empty. Zero-trans influx and efflux were about equivalent and so were zero-trans influx and equilibrium exchange flux. The apparent Michaelis-Menten constant and max velocity were about 500 uM and 100 pmol/s per ul cell H2O, respectively. The time courses of accumulation of radioactively labeled hypoxanthine at a concentration above the Michaelis-Menten constant differed noticeably in zero-trans and equilibrium exchange mode, but computer simulations showed that the difference is predicted by the symmetrical carrier model and does not reflect trans-stimulation. (Author abstract) (18 Refs)

51. EFFECT OF GLUCAGON ON AMINO ACID TRANSPORT AND CYCLIC ADENOSINE 3':5'-MONOPHOSPHATE PRODUCTION IN RAT HEPATOMA CELL LINE MCA-RH 8994 IN CULTURE.

Kelley DS
Dept. Biochemistry, West Virginia Univ., Sch. Medicine, Morgantown, WV, 26506
Cancer Res; 42(8):3116-3119 1982

The rates of alpha-aminoisobutyrate (AIB) transport and cyclic adenosine 3':5'-monophosphate (cAMP) production in response to glucagon (2 x 10(-7) M) have been determined in monolayer cultures of hepatoma cell line 8994 (refes to hepatoma cell line McA-RH 8994) under three different calcium treatments. The calcium treatments were: (a) control, cultures maintained in calcium-containing medium without prior calcium depletion; (b) calcium depleted, cultures maintained in calcium-free medium after treatment with ionophore A23187 and ethylene glycol bis(beta-aminoethyl ether)-N,N'-tetraacetic acid; (c) calcium repleted, cultures maintained in calcium-containing medium after treatment with A23187 and ethylene glycol bis (beta-aminoethyl ether)-N,N'-tetraacetic acid. In the control cultures, glucagon in the absence of methylisobutylxanthine (MIX) has no effect on the rate of AIB transport. AIB transport is approx doubled when glucagon is added in the presence of MIX. Calcium depletion and calcium repletion enhance the rate of AIB transport by about 50 and 100%, respectively. Glucagon and N6,O2-dibutyryl cyclic adenosine 3';5'-monophosphate (1 x 10(-4) M) further increase the rate of AIB transport by 10% in calcium-depleted cultures and by 50% in calcium-repleted cultures. The stimulation of AIB transport by glucagon as well as by the ionophore can be blocked by the simultaneous addition of cycloheximide (0.1 mM) or actinomycin D (3.2 uM). In the absence of MIX, there is about a two-fold increase in the level of cAMP in response to glucagon. In the presence of MIX, glucagon causes more than 100-fold increase in the level of cAMP. The presence or absence of calcium does not affect the basal or glucagon-stimulated production of cAMP in these cultures. We have reported previously that, in monolayer cultures of rat hepatocytes, calcium repletion enhanced the glucagon stimulation of AIB transport while it markedly inhibited the production of cAMP in response to glucagon. It is suggested that calcium rather than cAMP has a direct effect on the amino acid transport system. (Author abstract) (24 Refs)

MEMBRANE BIOGENESIS AND METABOLISM

52. CARBOXY TERMINUS OF POLYOMA MIDDLE-SIZED TUMOR ANTIGEN IS REQUIRED FOR ATTACHMENT TO MEMBRANES, ASSOCIATED PROTEIN KINASE ACTIVITIES, AND CELL TRANSFORMATION.

Carmichael GG, Schaffhausen BS, Dorsky DI, Oliver DB, Benjamin TL
Dept. Microbiology, Univ. Connecticut, Sch. Medicine, Farmington, CT, 06032
Proc Natl Acad Sci USA; 79(11):3579-3583 1982

We have constructed a transformation-defective polyoma virus mutant (Py 1387-T) that directs the synthesis of a normal small tumor antigen, a functional large tumor antigen, and a truncated (51,000-dalton) middle-sized tumor (mT) antigen that lacks 37 amino acids at its COOH terminus. The shortened mT polypeptide is missing the hydrophobic 'tail' thought to be responsible for the anchorage of this protein into the plasma membrane and is in fact in cytosol fractions. This truncated mT polypeptide is inactive in an in vitro protein kinase assay and is altered in its phosphorylation in vivo. Mutant 1387-T differs from wild-type virus in having a T-A base pair instead of a C-G base pair at nucleotide position 1387. This change was introduced into viral DNA by using a synthetic undecanucleotide as a specific mutagen. Wild-type polyoma DNA was rendered single stranded by molecular cloning into coliphage M13. The oligonucleotide, which hybridizes with a mismatch at the site to be altered, was used to prime the synthesis of double-stranded closed circular DNA. Progeny recombinant phages were screened by DNA sequence analysis for the desired base change. The polyoma mutant was reconstructed from recombinant phage replicative form DNA molecules containing the mutation. (Author abstract) (45 Refs)

53. LOSS OF SURFACE-BOUND ANTIBODY ACCOMPANYING THE ANTI-COMPLEMENTARY MODULATION OF LEUKEMIC B CELL IMMUNOGLOBULIN: CONTRASTING EFFECTS OF ANTIBODIES DIRECTED AGAINST IDIOTYPIC AND CONSTANT REGIONS.

Gordon J, Anderson VA, Stevenson GT
Dept. Tumor Biology, Karolinska Inst., Box 60400, S-104 01 Stockholm, Sweden
J Immunol; 128(6):2763-2769 1982

Guinea pig L2C leukemia lymphocytes display at their surfaces monoclonal IgM, which when complexed with antibody undergoes rapid redistribution and variable endocytosis. One consequence of this is that the cells can prove resistant to lysis by complement subsequently added to the system, a process termed here anti-complementary modulation. We studied quantitatively the extent of antibody loss accompanying the modulation by radioimmunolabeling the cell surfaces with 125I-Fab'-gamma fragments from an anti-antibody. Antibody directed against the constant region of the IgM light chain (anti-lambda) gave modulation effective against syngeneic (guinea pig strain

2) complement that closely paralleled the disappearance of anti-lambda from the cell surfaces. Antibody directed against the idiotypic region of the light chain (anti-Id) was as effective as anti-lambda in modulating against syngeneic complement. However, the bulk of the anti-Id was seen by radioimmunolabeling to persist on the surfaces of the resistant cells, even after prolonged exposure at 37 C, and was shown by immunofluorescence to be in a patched configuration. In contrast to the results with syngeneic complement, modulation effective against rabbit complement appeared to have an absolute requirement for clearing of the antibody: thus anti-lambda could modulate, anti-Id could not. The differences observed between anti-lambda and anti-Id could not be accounted for by differences in their isotypic (Ig subclass) composition nor by the numbers of antibody molecules bound. Studies with directly fluoresceinated and 125I-labeled and anti-lambda revealed endocytosis rather than shedding was the major route of antibody loss from the cell surfaces over the period of anti-complementary modulation. The findings are discussed in relation to mechanisms that enable leukemic B lymphocytes to escape destruction when confronted by antibody and complement. (Author abstract) (32 Refs)

54. NOVEL VESICULAR EXTRUSIONS DURING CELL SPREADING.

Rajaraman R, Westermark B, Ponten J, Vaheri A
Dept. Medicine, Dalhousie Univ., Halifax, Nova Scotia B3H 4H7, Canada
Experientia; 38(5):556-557 1982

During the late stages of cell spreading in vitro, the cells extrude a vesicular material into the medium. This phenomenon was observed in human glia and glioma cells as well as in human diploid fibroblasts MRC-5 and WI-38 cells. This extrusion of vesicular material is inhibited by cytochalasin-B and colcemid suggesting the involvement of microfilaments and microtubules and the active nature of this event. It appears that the cells may be excreting damaged surface components by a mechanism similar to patching, capping, and endocytosis. (Author abstract) (4 Refs)

55. VIDEO TIME-LAPSE MICROSCOPY OF PHAGOCYTOSIS AND INTRACELLULAR FATE OF CRYSTALLINE NICKEL SULFIDE PARTICLES IN CULTURED MAMMALIAN CELLS.

Evans RM, Davies PJ, Costa M
Dept. Pharmacology, Univ. Texas Medical Sch. at Houston, Houston, TX, 77025
Cancer Res; 42(7):2729-2735 1982

The endocytosis and intracellular distribution of carcinogenic crystalline nickel sulfide (NiS) particles in Chinese hamster ovary cells were studied using time-lapse video recording with phase-contrast and bright-field optics. Crystalline NiS particles were phagocytosed by Chinese hamster ovary cells in regions of membrane ruffling. While these particles may remain bound to the cell surface for variable time intervals (min to hr), their internalization generally required only 7 to 10 min. Endocytosed crystalline NiS particles exhibited saltatory motion, and lysosomes were observed to interact repeatedly with the particles in a manner similar to that observed during the digestion of macropinosomes. Particles were never observed to be exocytosed from the cell, and with time, most of the internalized particles aggregated in the region around the nucleus. After 24 to 48 hr, particle saltation decreased to a point where the particle position became relatively fixed in the perinuclear region, and in some instances, this was associated with a conspicuous vacuole formation around the particles. It is concluded that the uptake and distribution of crystalline NiS particles occur by normal endocytic and saltatory processes as occur during the formation and breakdown of macropinosomes. The observed lysosomal interaction with phagocytosed cytoplasmic NiS may accelerate particular nickel dissolution allowing entry of ionic nickel into the nucleus. (Author abstract) (30 Refs)

56. INHIBITION OF CELLULAR IMMUNITY IN SMALL CELL BRONCHOGENIC CARCINOMA OF THE LUNG: CHARACTERIZATION OF LOW-MOLECULAR SERUM FACTORS WHICH INHIBIT PROTEIN SYNTHESIS IN NORMAL LYMPHOCYTES (MEETING ABSTRACT).

Manke HG, Drings P, Lenhard V, Dirks HP, Toomes H
Onkologisches Zentrum Heidelberg-Mannheim, Mannheim, W. Germany
Onkologie; 4(5):266 1981

The synthesis of RBC-receptor proteins, a differentiation marker of human peripheral T-lymphocytes, is inhibited in patients with small cell bronchogenic carcinoma. This glycoprotein, which has been briefly characterized by Owen and Fanger as well as by Gurtler persists in the membrane for different periods of time depending on the T-cell population. When protein synthesis, nucleocytoplasmic mRNA transfer and mRNA synthesis are inhibited, normal T-lymphocytes are become depleted of RBC-receptor protein with characteristic temporal kinetics. In small cell bronchogenic carcinoma there is a very profound inhibition of the cellular immune system. In a study of 32 patients with small cell bronchogenic carcinoma the defect in cellular immunity correlated with a decrease in RBC-rosette forming cells and patient lymphocyte proliferation in response to mitogenic stimulation. Serum and serum fractions from patients with low RBC-rosette formation inhibited the expression of RBC-receptor protein by normal human peripheral lymphocytes within 60 min when incubated at 37C. This inhibition coincided with a reduction in protein synthesis (H3-amino acid uptake). The inhibitory serum fraction was further purified by means of high pressure liquid chromatography; the inhibitory trypsin-sensitive fraction lies in the 20,000 dalton region. The present studies refer to an inhibition of protein synthesis possibly on account of induction of a cyclic AMP-dependent protein kinase. (no Refs) (In GER)

57. EFFECTS OF CYCLIC NUCLEOTIDES ON THE SHEDDING OF TUMOR CELL SURFACE MEMBRANES.

Liepins A, De Harven E
Memorial Univ. Newfoundland, Faculty of Medicine, St. John's, Newfoundland, Canada
Exp Cell Res; 139(2):265-273 1982

Mastocytoma P815 cells can be induced to shed membrane vesicles (MV) from their surfaces by low temperature, a condition known to disrupt microtubules. In the present study we have investigated the effects of various nucleotides on this tumor cell surface behavior. Our data show that the cyclic nucleotides cAMP and cGMP significantly inhibit the low temperature-induced shedding of MV from the tumor cell surfaces. GTP and the phosphodiesterase inhibitor theophylline exhibited similar inhibitory effects. These data are interpreted in terms of nucleotide-induced stabilization of cytoskeletal components. Transmission (TEM) and scanning electron microscopy (SEM) of cells shedding MV revealed that these structures originated from blebs on the cell surface. (Author abstract) (32 Refs)

CELL ADHESION

58. CELLULAR INTERACTIONS IN THE CONTROL OF PROLIFERATION, MOVEMENT, AND ADHESION.

**International Cancer Research Data Bank Program Office
NCI, Westwood Building, Room 10A18, Bethesda, MD, 20205**
Cellular Interactions in the Control of Proliferation, Movement, and Adhesion (Oncology Overview) Steinberg, M, ed This Title is Available Through National Technical Information Service, Springfield, VA, as NTISUB/PB81-923005:; 76pp. 1981

ONCOLOGY OVERVIEWS are a service of the International Cancer Research Data Bank (ICRDB) Program of the National Cancer Institute. They are retrospective bibliographies containing 100-500 selected abstracts of published research on topics of high interest to cancer investigators. Included in the scope of this overview are several types of cell interactions which are impaired by neoplastic transformation. The abstracts cover the broad topics of cell proliferation, cell contact inhibition of locomotion, and cell adhesion. Within each of these areas specific subheadings deal with the effects of endogenous and exogenous agents on cell interaction, as well as the modification of cellular properties involved in the interactions between cells. There is also a section which contains theoretical models and general review articles.

59. MONOCLONAL ANTIBODY AGAINST HUMAN FIBRONECTIN WHICH INHIBITS CELL ATTACHMENT.

**Schoen RC, Bentley KL, Klebe RJ
Life Sciences - 3, Los Alamos Natl. Lab., Los Alamos, NM, 87545**
Hybridoma; 1(2):99-108 1982

Monoclonal antibodies to fibronectin were raised which inhibited specific biological functions of the fibronectin molecule. Hybridomas constructed between antigen-primed BALB/c mouse spleen cells and the BALB/c mouse myeloma P3-X63-Ag8 were screened. Monoclonal antibodies were isolated that recognized at least nine antigenic determinants of human fibronectin and at least two antigenic determinants of bovine fibronectin. Cross-reactivity of these antibodies with a wide range of mammalian species and sensitivity to various denaturing conditions are reported. (30 Refs)

60. EXPRESSION OF ADHESION-RELATED MEMBRANE COMPONENTS IN ADHERENT VERSUS NONADHERENT HAMSTER MELANOMA CELLS.

**Knudsen KA, Damsky CH, Buck CA
Wistar Inst. Anatomy and Biology, Philadelphia, PA, 19104**
J Cell Biochem; 18(2):157-167 1982

Nonadherent variant of hamster melanoma cell line RPMI 3460 was analyzed and its involvement in cell-substratum adhesion investigated. Two variants of RPMI 3460 were used: adherent line CM1 (cell monolayer) and nonadherent line CS1 (cell suspension). CM1 cells could be reversibly rounded and detached from the substratum with either of two antisera raised against material from hamster fibroblasts. Extracellular matrix-associated components did not induce the nonadherent cells to adhere to, or to spread on the substratum. However, continuous exposure to 2 x 1-(-6) M 5-bromodeoxyuridine (BudR) in dark induced a proportion of the cells to adhere to, and to spread on the substratum. Results showed that 10-fold more nonadherent cells than adherent cells were required to remove 50% of the rounding activity from anti-GP serum. NP40 detergent extract of the adherent cells contained approx 14x more total blocking activity than the extract from nonadherent cells. On the other hand, the NP40 extract of cells induced to adhere with BudR expressed similar blocking activity to that extracted from the normally adherent cells. Samples from adherent and BudR-treated cells contained, respectively, 5.5 and 16x more blocking activity than the sample from nonadherent cells. The presence of both blocking activity and the adherent phenotype correlated best with the presence of glycoproteins in the mol wt region of 120,000-140,000. (19 Refs)

61. EFFECT OF BASEMENT MEMBRANE PROTEIN LAMININ ON GROWTH AND DIFFERENTIATION OF AN EMBRYONAL CARCINOMA CELL LINE (MEETING ABSTRACT).

**Darmon M
Department de Biologie Moleculaire, Institut Pasteur, 25 rue du Dr. Roux, Paris 75015, France**
In Vitro; 18(3,part2):274 1982

Culture of cells in hormonally-defined media has allowed us to obtain physiological responses from cells usually unable to express them under non-defined conditions, and to study the effects on growth and differentiation of hormone-like factors and attachment factors. The embryonal carcinoma line 1003 is multipotential in vivo but unable to differentiate when grown in vitro in serum-containing medium. However, in a defined medium containing insulin, transferrin, selenium and fibronectin, 1003 cells grow for several generations and differentiate into neurons and embryonic mesenchyme. Under such conditions satisfactory cultures can only be obtained if cells are plated at a minimal density of 10(4)/cm2. In the present work fibronectin was replaced by laminin. It was found that cells attached and spread better, grew faster and could be plated at lower densities. The differentiation pattern was also altered: neurite extension was increased but, principally it was noted that an important induction of muscular differentiation occurred in cultures obtained after seeding the cells at low densities. Multinucleated myotubes obtained under these conditions could be stained with antibodies directed against embryonic muscle-specific myosin. The physiological significance of these results is discussed in the light of the fact that laminin is the major attachment protein found in the first basement membrane appearing in the embryo. (1 Ref)

62. PURIFICATION AND CHARACTERIZATION OF A COLLAGENOUS PROTEIN SECRETED BY A MURINE TERATOCARCINOMA-DERIVED CELL LINE.

**Ko CY, Priest RE
Dept. Pathology, Lab. Medicine, Emory Univ., Atlanta, GA, 30322**
Biochim Biophys Acta; 720(3):288-294 1982

A collagenous protein could be precipitated by (NH4)2SO4 from the culture medium of a murine teratocarcinoma-derived cell line. Further purification of this protein was achieved by combining DEAE-cellulose chromatography with either CM-cellulose or molecular sieve chromatography. The collagenous polypeptides had subunit mol wt of 160,000, if determined by molecular sieve chromatography, or 190,000, if determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and they are not linked by disulphide bridges. Amino acid composition of this collagen is similar to that of a murine Type IV collagen isolated from EHS sarcoma. The most prominent peptides resulting from cleavage of the protein by CNBr had estimated mol wt of 25,000, 23,000, 11,700 and 9,400. Pepsin treatment of this collagen under non-denaturing conditions produced three major fragments having molecular wt of 70,000, 45,000 and 43,000. We conclude that the collagen secreted by the murine teratocarcinoma-derived cell culture is a Type IV

basement membrane collagen. Therefore, this culture system should provide a continuous source of Type IV collagen, which may be used to study the interaction of this collagen with other basement membrane components. (Author abstract) (22 Refs)

63. MECHANISM OF TRANSFORMATION BY ROUS SARCOMA VIRUS: EVENTS WITHIN ADHESION PLAQUES.

Rohrschneider L, Rosok M, Shriver K
Fred Hutchinson Cancer Res. Center, Seattle, WA, 98104
Cold Spring Harbor Symp Quant Biol; 46(part2):953-965 1981

Using several mutants of Rous sarcoma virus which contain partial lesions in the src gene, various molecular events within adhesion plaques of infected chicken embryo cells were examined in order to determine their contribution to the transformed phenotype. The disposition of pp60v-src, vinculin, fibronectin, and stress fibers and the phosphorylation of vinculin on tyrosine within chicken embryo cells infected with partial-transformation mutants are diagrammed. (32 Refs)

64. MOUSE ROSETTE (M ROSETTE) IN THE DIFFERENTIAL DIAGNOSIS OF LYMPHOPROLIFERATIVE DISORDERS.

Gabrielli A, Leoni P, Marchegiani G, Montillo M, Centurioni R, Montroni M, Danieli G
Istituto di Patologia Speciale Medica e Metodologia Clinica, Universita di Ancona, Ancona, Italy
Minerva Med; 73(20):1383-1390 1982

The capacity of a subpopulation of human B lymphocytes to form nonimmune rosettes with mouse red blood cells (M rosettes) was used for the differential diagnosis of B-cell lymphoproliferative diseases in 24 patients (12 men and 12 women, 51-77 yr old) with chronic lymphocytic leukemia (CLL), 7 with leukemic lymphoma (LL) and 3 with hairy-cell leukemia (HCL). Four of the 24 patients CLL were in Stage 0, 16 in Stages I-II and 4 in Stages III-IV. The percentages of M rosettes were distinctly increased in the patients with HCL and LL. The percentage of M rosettes remained extremely elevated at lymphocyte counts below 10,000/cubic mm. The absolute counts of lymphocytes carrying this membrane receptor was found to increase with increasing lymphocyte count, which is an expression of the neoplastic population, and with the advancement of the clinical stage according to Rai's classification. (11 Refs) (In ITA)

65. EXPRESSION OF COLONY STIMULATING ACTIVITY (CSA) AND PRODUCTION OF COLONY FORMING UNIT (S) (CFU-C) BY ACUTE MYELOID LEUKAEMIA CELLS SHOWING MATURATION IN SHORT-TERM CULTURE.

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Istituto di Microbiologia dell'Universita di Parma, via Gramsci, 14-43100 Parma, Italy
Haematologica (Pavia); 67(2):188-198 1982

Relationship of colony stimulating activity (CSA) and colony forming unit/culture (CFU-c) to phenotypic features of the in vitro maturation process was studied in three cell populations taken directly from patients with hematologically proven acute myelocytic leukemia. Patients had no prior chemotherapy and had greater than 90% definite blast cells in peripheral blood. Relevant clinical data of the patients are presented. Cell cultures of the three patients showed almost identical growth patterns. After an initial decrease during the first 2-3 days, the number of cells increased seven-fold in the following wk, and progressively declined thereafter. Adherent cells from two patients behaved like and had the appearance of macrophages. The ability of non-adherent cells to undergo maturation in culture was confirmed by the Fc receptors on the cell surface and the presence of esterases characterizing the differentiation of monocytes or granulocytes. In two patients, cells had a high proportion of CFU-c at the beginning of the culture period (40/2 x 10(5) and 24/2 x 10(5) cells, respectively). The number of CFU-c decreased to a much lower level in both patients by day 10, followed by a secondary increase, more pronounced in one patient. Measurable levels of CSA were produced by all three non-adherent cell populations. However, the greatest activity was produced by the isolated adherent cell population of the two patients 14 days after culture and near the time of the secondary increase in CFU-c. In cell populations unable to produce phenotypic changes characteristic of maturation, both CFU-c and CSA were absent. (20 Refs)

OTHER RELATED STUDIES

66. METASTASIS: AN OVERVIEW.

Zeidman I
Dept. Pathology, Sch. Medicine, Univ. Pennsylvania, Philadelphia, PA
Cancer Biol Rev; 2:1-27 1981

Current controversies and new concepts in the study of metastasis are reviewed. Metastasis via the bloodstream, metastasis in the lymphatic system, and the nature of the cancer cell surface and the immune response in relation to metastasis are discussed. Acceptability of the experimental metastasis model, the importance of 'homing,' and the roles of the regional lymph node, and general immune response in checking cancer remain controversial. (111 Refs)

67. CANCER-DEPENDENT HYDROPHOBICITY CHANGES IN MEMBRANES OF PHYTOHAEMAGGLUTININ STIMULATED LYMPHOCYTES.

Pritchard JA, Siddall JE, Bater AJ, Kerby IJ, Sutherland WH, Deeley TJ
Immunology Unit, Radiation Science Laboratories, Velindre Hosp., South Wales Radiotherapy and Oncology Service, Whitchurch, Cardiff CF4 7XL, Wales
IRCS Med Sci [Cancer]; 9(8):711 1981

Fluorescence intensity changes were investigated in phytohemagglutinin (PHA) stimulated lymphocytes from 12 healthy donors and 11 patients with histologically proven carcinoma of the cervix. Statistically significant increases in fluorescence of N-phenyl-1-naphthylamine were found in all PHA stimulated cell suspensions from healthy donors, significant decreases were found in 8/11 cancer patients, and no significant response was seen in 3/11 patients. These findings support the hypothesis that there are significant differences between normal lymphocytes and those from tumor-bearing hosts. (7 Refs)

68. CELLULAR MECHANISMS OF ANTICANCER DRUG RESISTANCE (MEETING ABSTRACT)

Seeber S
Innere Klinik und Poliklinik, westdeutsches Tumorzentrum, Hufelandstrasse 55, D 4300 Essen, W. Germany
J Cancer Res Clin Oncol; 103(Suppl):A51 1982

The cellular mechanisms for resistance depend on the cell line studied and the drug used. They include 1) impaired transport of the agent into the tumor cell, 2) impaired intracellular activation, 3) decreased intracellular retention, (by increased enzymatic degradation or deletion of binding proteins of active outward extrusion), 4) increased intracellular concentration of target enzymes, 5) alterations in tertiary structures of target enzymes, 6) adoption of alternative metabolic pathways by the tumor cells, 7) repair of DNA damage, 8) changes in the distribution of receptor proteins at the cell surface, cytoplasm or nucleus, 9) immunological mechanism leading to inactivation of antigenic cytotoxic substances and finally, 10) alterations of tumor cell kinetics with shortening of drug sensitive phases or decrease in the proliferating tumor cell pool. Resistance of a tumor as experienced clinically may already occur after a minor reduction of cell sensitivity. In order to define the underlying mechanisms of resistance, especially with regard to new anticancer substances, model investigations with very high levels of resistance to various actual drugs may be helpful. In the author's laboratory, such a model was established 4 years ago using the in vivo propagation of an Ehrlich ascites tumor primarily sensitive to most anticancer agents (EATWT) from which sublines resistant to either anthracyclines (EATDNM), cisplatin (EATDDP), etoposide (EATVP) or daunorubicin plus cisplatin (EATDNM/DPP) have been developed by weekly transplantations and biweekly drug exposure. These lines are being analyzed biochemically and the pharmacology of the drugs is studied at a cellular level. (no Refs)

69. USE OF FLUORESCENT PROBE FOR DETECTION OF DIFFERENCES OF LEUKOCYTE MEMBRANES IN CHRONIC LEUKEMIAS.

Baglaev TN, Kalinin NN, Dobretsov GE, Kosankova ST, Vladimirov IuA
Dept. Biophysics, N. I. Pirogov Second Medical Inst., Moscow, USSR
Biull Eksp Biol Med; 93(4):35-37 1982

To evaluate the differences between the membranes of normal lymphocytes and leukemic cells, peripheral blood leukocytes from 6 patients with chronic myeloid leukemia (CML), 5 patients with chronic lymphoid leukemia (CLL), and 12 healthy controls were stained with the fluorescent probe 3-methoxybenzanthrene. The mean intensity of fluorescence in CML (71.0 units) was significantly greater than that in CLL (34.0) and in healthy controls (32.0). (9 Refs) (In RUS)

70. USE OF FLUORESCENT PROBES FOR THE STUDY OF THE CHANGES OF CELL MEMBRANES OF CHINESE HAMSTER FIBROBLASTS AFTER LASER AND X-RAY IRRADIATIONS.

Abdvakhitova AK, Parkhomenko IM, Sokolova TN
Faculty Biology, M. V. Lomonosov State Univ., Moscow, USSR
Radiobiologia; 22(2):155-159 1982

The effect of laser and x-irradiations on the structure of cell membranes was studied in cultured Chinese hamster fibroblasts. Analysis of the binding of the fluorescent probe showed 2-3x decrease of the quantum yield of the fluorescence. Laser irradiation resulted in a 1.7-1.8x decrease of the constant of binding, compared with 1.5-1.7x increase after x-irradiation. (11 Refs) (In RUS)

71. STIMULATION OF FLUORESCENCE IN A SMALL CONTACT REGION BETWEEN RAT BASOPHIL LEUKEMIA CELLS AND PLANAR LIPID MEMBRANE TARGETS BY COHERENT EVANESCENT RADIATION.

Weis RM, Balakrishnan K, Smith BA, McConnell HM
(c/o McConnell) Dept. Chemistry, Stanford Univ., Stanford, CA, 94305
J Biol Chem; 257(11):6440-6445 1982

We have included a lipid hapten in lipid monolayers coated on alkylated quartz microscope slides to serve as specific antibody-dependent targets for rat basophil leukemia cells. At room temperature basophils armed with specific monoclonal IgE antibodies adhere tightly to the monolayer only when the monolayers contain lipid hapten and only in the presence of Mg++ and/or Ca++. The basophils undergo specific IgE- and hapten-dependent immunologic degranulation under these conditions. Fluoresceinated IgE antibodies bound to Fc receptors on the basophil and located within approx 100 nm of the monolayer target fluoresce when excited by laser radiation undergoing total internal reflection within the quartz slide. An optical interference pattern produced by two focused intersecting coherent laser beams having total internal reflection within the quartz slide serves as a control to ensure that all of the emitted fluorescence radiation from the IgE molecules is due to stimulation by evanescent radiation and not by scattered radiation. The interference pattern is also used for pattern photobleaching experiments to determine the rates of lateral diffusion of IgE molecules bound to the monolayers, in the absence of cells. Specific antibody-dependent adherence of basophils to the monolayer target results in a marked enhancement of IgE fluorescence in the field of the evanescent wave. The concentration of IgE molecules in the contact region appears to be high and could give rise to Fc-Fc receptor complex formation or other redistributions of Fc receptors that provide signals for basophil degranulation. (Author abstract) (25 Refs)

72. BIOPHYSICAL IDENTIFICATION AND SORTING OF HIGH METASTATIC VARIANTS FROM B16 MELANOMA TUMOR.

Lessin SR, Abraham SR, Nicolini C
(c/o Nicolini) Div. Biophysics, Dept. Physiology/Biophysics, Temple Univ. Health Sciences Center, 3200 North Broad St., Philadelphia, PA, 19140
Cytometry; 2(6):407-413 1982

Characterization of B16-F1 (low) and B16-F10 (high) variants of B16 melanoma by flow cytometry with cell sorting and scanning cytometry is described. Parameters studied included (a) viably staining B16-F10 and B16-F1 with fluorescamine and counterstaining with ethidium bromide, (b) detecting differential fluorescamine uptake between B16-F1 and B16-F10 to identify and sort selected subpopulations which could be replated for in vivo assay of metastatic potential, and (c) scanning cytometry (densitometric-geometric image analysis) of Feulgen-stained 'sorted' B16-F10 subpopulations. Cell viability and differential fluorescamine uptake were found to be a function of the dye to cell ratio. Differential uptake and decreased viability induced by 30 mg fluorescamine/5 x 10(6) cells was due both to a complex interaction between ethidium bromide and fluorescamine and to the saturation of primary amine sites which in B16-F10 cells occurred at high concentrations. When a subpopulation of B16-F10 (B16-F10M-b) was sorted and tested in vivo, the cells were observed to have an increased metastatic potential compared to the parent cells. F10M-b's ability to produce more pulmonary metastases than its parent was indicative of fluorescamine's capacity to be used as a mem-

brane probe to identify specific membrane organizations related to enhanced metastatic potential. The relationship of membrane organization and chromatin configuration to metastatic growth rate is discussed. (31 Refs)

73. PLASMINOGEN ACTIVATOR, FIBRONECTIN, LYMPHOTOXIN SENSITIVITY, AND NATURAL SKIN REACTIVITY RELATIONSHIPS TO GUINEA PIG CELL TUMORIGENICITY.

McCabe RP, Evans CH
Tumor Biology Section, Lab. Biology, Div. Cancer Cause and Prevention, NCI, NIH, Public Health Service, U.S. Dept. Health and Human Services, Bethesda, MD, 20205
JNCI; 68(2):329-336 1982

The quantitative expression of five properties of chemical carcinogen-induced, neoplastically transformed NIH strain 2 guinea pig fibroblasts was compared in cells possessing thousandfold differences in tumorigenicity. Plasminogen activator synthesis, sensitivity to lymphotoxin inhibition of cell proliferation, and the ability to induce a natural delayed tuberculin-type skin reaction in nonimmune syngeneic guinea pigs correlated directly with the number of cells required to produce a tumor. The most tumorigenic cells (10(2)-cell threshold dose) produced the most plasminogen activator, were most sensitive to lymphotoxin, and produced the greatest skin reactivity. Cells with a threshold tumor dose of 10(5)-10(7) cells exhibited the lowest expression of these properties. Fibronectin incorporation into an extracellular matrix was diminished in tumorigenic cells, as was anchorage-dependent growth; but neither diminished fibronectin incorporation nor the decreased anchorage requirement correlated quantitatively with the number of cells required to produce a tumor. The present investigation indicates that plasminogen activator synthesis, sensitivity to lymphotoxin, and the capacity of tumorigenic cells to induce natural delayed-type skin reactivity are among the factors that influence initial tumor growth. Plasminogen activator, an extracellular protease, may aid in the growth and spread of tumor cells in vivo by interfering with host fibrin deposition and by inactivating other host proteins such as lymphotoxin. (Author abstract) (46 Refs)

74. ELECTRON SPIN RESONANCE STUDIES ON INTACT CELLS AND ISOLATED LIPID DROPLETS FROM FATTY ACID-MODIFIED L1210 MURINE LEUKEMIA.

Simon I, Burns CP, Spector AA
Dept. Biochemistry, Univ. Iowa Coll. Medicine, Iowa City, IA, 52242
Cancer Res; 42(7):2715-2721 1982

It has been suggested that the formation of cytoplasmic lipid droplets may produce an artifact and be responsible for the differences in membrane physical properties detected in lipid-modified cells using fluorescence polarization or spin label probes. To investigate this, the electron spin resonance spectra of lipid droplets isolated from the cytoplasm of L1210 leukemia cells were compared with spectra obtained from the intact cell. Mice bearing the L1210 leukemia were fed diets containing either 16% sunflower oil or 16% coconut oil in order to modify the fatty acid composition of the tumor. A microsome-rich fraction prepared from L1210 cells grown in animals fed the sunflower oil-rich diet contained more polyenoic fatty acids (52 versus 29%), while microsomes from L1210 cells grown in animals fed the coconut oil-rich diets contained more monoenoic fatty acids (37 versus 12%). The order parameter calculated for lipid droplets labeled with the 5-nitroxystearic acid spin probe was only about one-half that of intact cells, whereas it was similar to that obtained for pure triolein droplets suspended in buffer. Order parameters of the inner hyperfine splittings calculated from the spectra of cells grown in the sunflower oil-fed animals [0.543 +-0.001 (SE)] were lower than those from the cells grown in animals fed the coconut oil diets (0.555 +-0.002) (p less than 0.005). In contrast, the order parameters of the lipid droplets isolated from the cells grown in animals fed sunflower oil (0.303 +-0.029) or coconut oil (0.295 +-0.021) were not significantly different, indicating that motion of a spin label probe in the highly fluid cytoplasmic lipid droplets is not affected by these types of modifications in cellular fatty acid composition. Therefore, the electron spin resonance changes that were observed in the intact cells cannot be due to localization of the probe in cytoplasmic lipid droplets. These results support the conclusion that the electron spin resonance changes observed with the 5-nitroxystearic acid spin probe are due to changes in membrane fluidity produced by the modification in cellular lipid composition. (Author abstract) (31 Refs)

75. ELECTRON SPIN RESONANCE STUDIES OF MEMBRANE FLUIDITY OF NORMAL AND MALIGNANT HUMAN NEURAL TISSUES.

Wersto RP
Loyola University of Chicago, Chicago, IL
Diss Abstr Int [B]; 42(12,pt1):4660-B 1982

The correlation between membrane fluidity phospholipid headgroup and acyl side chain composition with tumorigenicity was examined using an in vitro model derived from normal and malignant human brain cells. The system is composed of normal glial cells (NFB line) and five tumor-derived cell lines, one of which, the SA101 line from a glioma, presents in vitro culture characteristics and membrane lipid changes comparable to cultured human glia. Among the remaining lines, two were cultured from medulloblastoma (E1202 and TE907), whereas two were initiated from gliomas (SA4 and 251 MG 3C lines). Colon-forming efficiency and growth in athymic nude mice ascertained the malignant potential of the SA4 and E1202 lines and the non-tumorigenicity of the SA101 TE907 cells. Although the 251 MG 3C cells did not produce tumors in nude mice, they possessed a substantial soft agar colony-forming efficiency. Membrane lipid alterations reveal a correlation of in vivo and in vitro tumorigenicity with phospholipid headgroup class composition rather than phospholipid acyl side chain unsaturation or mean chain length. Membrane fluidity was investigated by electron spin resonance using the 5-, 12-, and 16-doxystearate spin labels. For all three probes, the SA4 and E1202 tumorigenic lines possess an increase in fluidity above the level of the NFB line, with the largest absolute increases being observed as the spin label position is moved towards the bilayer anterior. All cell lines generate a flexibility gradient, indicating that this common denominator of membranes is not altered in neoplasia. Evidence is also presented which indicates that malignant neural cell lines possess increased (phosphatidylcholine + spingomyelin)/(phosphatidylethanolamine + phosphatidylserine + phosphatidylinositol) ratios. (no Refs)

76. HEMATOLOGY.

Beck WS
Harvard Medical Sch., Boston, MA
Hematology. Cambridge, MA, The MIT Press, 462 pp., 1981.

Hematology is discussed with emphasis on physiology and pathophysiology. Therapy is discussed as it relates to pathophysiology. The following topics are discussed. Hematopoiesis

and introduction to the anemias; reticuloendothelial system, lymphatic system, and spleen; normocytic anemias; megaloblastic anemias (vitamin B12 deficiency, folic acid deficiency); hypochromic anemias (iron deficiency and excess, heme metabolism and the porphyrias); Hb structure and function; Hb in sickle cell anemia and other hemoglobinopathies; the thalassemias; hemolytic anemias; immunohemolytic anemias; membrane and metabolic disorders and hemolytic anemias; physiology and blood groups; transfusion therapy and blood groups; physiology and WBC; phagocytosis and its disorders in relation to WBC; pathology of WBC; polycythemias; leukemias; clinical aspects and pathology of malignant lymphomas; plasma cell disorders and the dysproteinemias; histiocytoses and lipidoses; protein interactions in the clotting mechanism of hemorrhagic disorders; platelets and purpura in hemorrhagic disorders, and disorders of primary and secondary hemostasis in hemorrhagic disorders. (205 Refs)

77. CONTACT-STIMULATED PROLIFERATION OF CULTURED MOUSE EPIDERMAL CELLS BY 3T3 FEEDER LAYERS: INHIBITION OF PROLIFERATION BY 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA).

Miller DR, Hamby KM, Slaga TJ
(c/o Slaga) Univ. Tennessee-Oak Ridge Graduate Sch. Biomedical Sciences, Oak Ridge Natl. Lab., Oak Ridge, TN, 37830
J Cell Physiol; 112(1):76-82 1982

Mouse epidermal cells can be subcultured at 31 C onto an irradiated BALB/c 3T3 clone A31 feeder layer. A31 cells were specifically required for optimal production of keratinizing epidermal colonies in secondary culture. The effect was not transmitted through the medium nor by the culture surface. Contact with metabolizing A31 cells was probably necessary. 12-O-Tetradecanoylphorbol-13-acetate blocked epidermal colony formation. The A31-epidermal cell interaction is apparently not a typical mesenchymal-epithelial interaction, since the basement membrane would prevent this contact in intact skin. (61 Refs)

78. GRADING OF BRAIN TUMORS BY A LYSOSOMAL FRAGILITY TEST.

Fabiani A
Dept. Neuropathology, Univ. Turin, Via Cherasco, 15, I-10126 Torino, Italy
Zentralbl Neurochir; 43(1):35-42 1982

The stability of lysosomes is a function of osmotic pressure, temperature and time. Detailed studies have shown that the release and availability of lysosomal enzymes changes according to the degree of membrane permeability. This paper describes a modification of the controlled temperature and osmotic freezing-sectioning technique for characterization of lysosomal stability in brain tumors. The method is based on the enzyme measurements of released acid phosphatase from sections of unfixed tissue under appropriate physical conditions. The results suggest the possibility of a relation between lysosomal stages of activation, enzyme latency and brain tumor malignancy. (Author abstract) (31 Refs)

79. ENHANCED SUSCEPTIBILITY OF GLUTARALDEHYDE-TREATED TUMOR CELLS TO ANTIBODY-DEPENDENT MACROPHAGE-MEDIATED CYTOLYSIS.

Iwata-Dohi N, Esumi-Kurisu M, Ikenami M, Sadatsune K, Mizuno D, Yamazaki M
(c/o Esumi-Kurisu), Teikyo Univ., Sagamiko, Kanagawa 199-01, Japan
Cancer Res; 42(8):3196-3200 1982

The effect of treatment of target tumor cells with glutaraldehyde (GA) on antibody-dependent macrophage-mediated cytolysis was investigated in a C3H/He mouse-MM46 syngeneic tumor system. GA-treated tumor cells were more susceptible to antibody-dependent macrophage-mediated cytolysis. Lysis of GA-treated cells was observed with less antibody and fewer macrophages than that of untreated cells. However, GA-treated cells had the same amount of antigen sites and the same activity of antibody-dependent attachment to macrophages as do fresh tumor cells. Thus, this enhancement of lysis did not occur at the antibody-binding step or the early step of contact between macrophages and tumor cells but at the subsequent step, perhaps at the tumor lysis step. GA-treated tumor cells could not synthesize DNA or RNA, but cells attenuated with mitomycin C or actinomycin D were not susceptible to antibody-dependent macrophage-mediated cytolysis. These results suggest that the cytostatic effect of GA does not involve change in susceptibility of target cells to lysis, but some impairment of the cell membrane. GA-treated tumor cells could not grow in vivo and could induce antitumor immunity. (Author abstract) (20 Refs)

80. STUDY OF THERMOINDUCED STRUCTURAL CHANGES IN MEMBRANES AND LIPIDS OF LYSOSOMES DURING EXPERIMENTAL CARCINOGENESIS.

Kotrikadze NG, Gabunilia GD, Dzhishkariani OS, Tsartsidze MA, Lomsadze BA
State Univ., Tbilisi, USSR
Biofizika; 27(3):454-457 1982

Structural changes in the membranes and lipids of lysosomes isolated from normal liver, liver of tumor-bearing rats, and Walker 256 carcinosarcoma were studied by means of spin probes. Analysis of rotational correlation for the spin probes showed that the structural transitions in the lysosomes of the normal liver occurred at 34-64 C, compared with 30 C for the from the liver of tumor-bearing rats and 28-40 C for those from the tumor. (8 Refs) (In RUS)

81. CARCINOGENESIS AND BIOLOGICAL EFFECTS OF TUMOR PROMOTERS.

Hecker E
Inst. Biochemistry, German Cancer Res. Center, Heiodelberg, W. Germany
Carcinogenesis 7: 664 pp., 1982.

The most recent experimental findings on the etiological, cell biological, and molecular facets of carcinogenesis are reviewed. The basic problems of whether specific carcinogens function as promoters of carcinogenesis, as stimulators of initiation, or both, are addressed with respect to in vivo animal studies, epidemiological studies, and in vitro studies of tumor promotion. Carcinogenesis and tumor promotion in vivo is discussed with respect to the skin as a target tissue and nonepidermal target tissues. Effects of tumor promoters on cell proliferation and differentiation are reviewed for the epidermis and

nonepidermal cell systems. Membrane interactions and receptors, effects on intercellular communication and cell metabolism, and viral and immunologic aspects are reviewed. Epidemiologic studies of esophageal cancer in Linxian County, China, nutritional carcinogenesis, and breast cancer as an example of promoting effects are discussed. (1368 Refs)

82. FUNDAMENTAL MECHANISMS IN HUMAN CANCER IMMUNOLOGY.

Saunders JP, Daniels JC, Serrou B, Rosenfeld C, Denney CB
University of Texas, Graduate Sch. Biomedical Sciences, Galveston, TX

Dev Cancer Res 5: 533 pp., 1981.

Human cancer immunology is considered under the following headings: cell membranes (modulation of complement 3b receptor synthesis on a human B lymphoblastoid cell line, membrane lipids and cellular functions in normal and malignant lymphocytes, tumor cell heterogeneity and blood-borne metastasis, relationship between cell surface thrombin receptors and cytoplasmic microtubules, and other topics), modulation of immune reactions (interferons, adoptive chemoimmunotherapy of murine leukemia with cells secondarily sensitized in vitro and cells numerically expanded by culture with interleukin 2; regulation of natural killer cell activity; specific and non-specific cellular interactions modulating host resistance to tumors; interrelationships between immunoregulatory cells, neoplastic diseases, and immunodeficiency states, and other subjects), surface antigens (possible clinical significance of circulating immune complexes in melanoma patients, natural and activated killer lymphocytes, monoclonal antibodies as probes for cell surface changes in human malignancy, quantitation of isotopic immunoglobulin therapy to tumor-associated proteins, and other topics), tumor surveillance (new approaches to specific and non-specific immunotherapy of established cancer metastases; immunomodulation by nutritional means; specific immune responses to chemically induced tumors; the ability of natural killer cells functions through recognition of glycoconjugates on target cell membranes; in situ, in vitro, and systemic regulation of natural killer cytotoxicity; and characteristics of natural killer cells and their possible role in resistance against tumor growth), and perspectives and prospects. (912 Refs)

83. ENUCLEATION ELIMINATES A DIFFERENTIATION-SPECIFIC SURFACE MARKER FROM NORMAL AND LEUKEMIC MURINE ERYTHROID CELLS.

Schlegel RA, Phelps BM, Cofer GP, Williamson P
Molecular and Cell Biology Program, Pennsylvania State Univ., University Park, PA, 16802
Exp Cell Res; 139(2):321-328 1982

Mammalian erythropoiesis includes a step in which the nucleus is extruded through the cell membrane. We have investigated the relationship between concanavalin A (conA) plasma membrane receptors, which are known to leave the incipient reticulocyte during enucleation, and regions of the plasma membrane which bind merocyanine 540, a differentiation-specific marker of hematopoietic cells. The distribution of these two fluorescent probes was examined on living cells from the spleens of neonatal mice and on erythroleukemia cells induced to enucleate in culture. In both cases, the region of the membrane extruded with the nucleus preferentially binds conA and merocyanine 540, whereas the plasma membrane which is left behind retains the capacity to bind another lectin, wheat germ agglutinin. The implications of these findings are discussed with respect to the mechanism by which markers are eliminated from the erythrocyte cell surface. (Author abstract) (12 Refs)

84. INDUCTION OF NEOPLASTIC CELL GROWTH BY QUIESCENT CELLS IS MEDIATED BY SERUM CONCENTRATION AND CAMP PHOSPHODIESTERASE INHIBITORS.

Bertram JS, Bertram BB, Janik P
Grace Cancer Drug Center, Roswell Park Memorial Inst., Buffalo, NY, 14263
J Cell Biochem; 18(4):315-538 1982

Studies have shown that for transformed mouse fibroblasts (C3H/101/2) and Lewis lung carcinoma model systems, the expression of transformation is conditional upon the permissive behavior of neighboring cells. Such permissive behavior could be due to a failure in intercellular communication or to a failure in signal reception or processing. For a constant plating density an increase in serum concentration was shown to cause a dose responsive decrease in N-methyl-N-nitro-N-nitrosoguanidine (MNNG)-induced transformation frequency in fibroblast cells. This increase in serum concentration produced an increase in saturation density of confluent cells. A model is developed which suggests that interference in communication between cells occurs during transformation. The passage of small growth inhibitory substances through gap junctions between contacting cells controls cell cycle phase. Autonomous growth would occur through attenuation of signal intensity. This studies have identified two possible factors which may control signal strength, ie, cell density and elevated cAMP. The model thus suggests that transformed cells are deficient in their ability to form communication channels with themselves or with adjacent normal cells. The major effect of phosphodiesterase inhibitors may be to increase the latent period between tumor injection and the appearance of tumor. Applications of the model to phosphodiesterase inhibitors and to tumor promotion are discussed. (42 Refs)

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DECEMBER, 1982

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BIOCHEMICAL ANALYSIS OF CELL SURFACE COMPONENTS

1. ENZYMATIC PROPERTIES IN PERICELLULAR MEMBRANES OF LEUKEMIC CELLS.

Losa G
Lab. Cellular Pathology, Ticino Inst. Pathology, Locarno, Switzerland
Adv Exp Med Biol; 145:377-384 1982

Enzyme characteristics of the plasma membrane were analyzed biochemically in cell populations isolated from the peripheral blood of leukemic patients to distinguish the various types of acute leukemias. Very low enzyme activities were generally recorded in cells of both non B/non T lymphoblastic and T leukemias. Differences in the activity of some enzymes were sufficient to allow a clear-cut differentiation of the two leukemic cell populations. In lymphoblastic leukemia high 5'-nucleotidase activity was measured with a mean level of approx 45 nanomole (nmol)/hr/10(6) cells. In some patients, the activity reached a mean value of 230 nmol/hr/10(6) cells, suggesting an earlier differentiation stage of the corresponding leukemic cells. Compared to the control mononuclear cell population, moderate decreased activities were measured for 5'-nu-

HE 20.3173/2:

cleotidase, alkaline phosphomonoesterase, and gamma-glutamyltranspeptidase. The activity of 5'-phosphodiesterase was significantly high in approx 40% of the patients while in the remainder it was practically undetectable or very low (range 0-2.5 nmol/hr/10(6) cells). The absence of activity of 5'-phosphodiesterase was inversely related to the expression of the immune associated antigen (Ia), whereas the absence of Ia correlated with the presence of high activity (range 3.8-16.8 nmol/hr/10(6) cells). A positive low level of terminal deoxynucleotidyl transferase activity was observed in approx 20% of patients but with an activity range (0-30 picomole/min/mg protein) and distribution similar for both subgroups of acute myeloid leukemia. (14 Refs)

2. CROSS-REACTING ANTIGENS ON L5178Y CELLS WHICH SERVE AS TARGETS FOR CYTOTOXIC T-LYMPHOCYTE LYSIS DURING ESTABLISHMENT OF THE TUMOR DORMANT STATE.

Weinhold KJ, Wheelock EF
(c/o Wheelock) Dept. Pathology and Lab. Medicine, Hahnemann Medical Coll., Philadelphia, PA, 19102
Cancer Res; 42(9):3607-3616 1982

We have attempted to identify the antigen on L5178Y cells that is the target for peritoneal cytotoxic T-lymphocytes (CTL) generated in L5178Y-O (original) immunized and challenged DBA/2 mice during establishment of the L5178Y cell tumor dormant state. These CTL exhibited in vitro lytic activity against methylcholanthrene-induced L5178Y and P815Y cells as well as Gross virus-induced BALB/c cells but not against a variety of other H-2d-tumor cell lines. The pattern of susceptibility to cell-mediated immune cytolysis was identical to the pattern of AB-dependent complement-mediated cytolysis produced by a rabbit anti-L5178Y antiserum. Quantitative expression of surface H-2d determinants was not a limiting factor in tumor cell lysis by CTL. The degree of CTL lysis of the susceptible cell lines was directly related to the amount of tumor-associated antigen expressed on the cell surface. The pattern of in vitro susceptibility to CTL lysis correlated well with in vivo transplantation resistance. The L5178Y cell antigen target for both CTL and Ra-anti-L5178Y serum lysis is likely to be either an endogenous AKR ecotropic viral glycoprotein with a mol wt of 71,000 or one of two cell surface determinants, at Mr 85,000 and 135,000. These higher-mol-wt antigens are neither endogenous AKR ecotropic viral-induced nor murine leukemia virus group-specific precursor structural proteins but may be transformation antigens shared by the susceptible tumor cell lines. (Author abstract) (37 Refs)

3. RESPONSES OF PRENEOPLASTIC EPIDERMAL CELLS TO TUMOR PROMOTERS AND GROWTH FACTORS: USE OF PROMOTER-RESISTANT VARIANTS FOR MECHANISM STUDIES.

Colburn NH, Wendel E, Srinivas L
Lab. Viral Carcinogenesis, NCI, Frederick, MD, 21701
J Cell Biochem; 18(3):261-270 1982

The mechanism of late stage promotion was studied in the JB6 mouse epidermal cell line. Second-stage promoters, including phorbol diesters which act both as first- and second-stage promoters, and mezerein, which acts primarily as a second-stage promoter, showed activity in promotion of transformation in JB6 cells. The second-stage antipromoter retinoic acid inhibited promotion of transformation by 12-O-tetradecanoylphorbol-13-acetate (TPA) but the antiproteases antipain and leupeptin, which inhibit first-stage promotion, did not, even at concentrations greater or equal to those that inhibited promotion of transformation in 10T1/2 cells. Three of four mitogen-resistant lines, R219, R6141, and R23, were promotable to anchorage independence by TPA. Thus, promotion of transformation by TPA in JB6 cells can occur without the release-from-quiescence of mitogenic response to TPA. Cross-resistance to mezerein and epidermal growth factor (EGF) occurred in two TPA promotion-resistant clones of JB6, C130, and C125. C125 and C130 showed moderate to high levels of EGF binding while three promotable lines, R219, R6141, and R23, showed little or no EGF binding. Thus, EGF binding is not a requirement for promotion of transformation in JB6 cells by TPA. The role of TPA-induced changes in the sialic-acid-containing plasma membrane glycolipids was investigated. TPA produced a 10-fold decrease in precursor incorporation into a trisialoganglioside and a 2-fold increase in incorporation into an unidentified ganglioside. These findings suggest that gangliosides may be important in promotion of tumor cell phenotype. A model for the sequence of events necessary for promotion of transformation in JB6 cells is proposed. (32 Refs)

4. UNIQUE CELL SURFACE GLYCOPROTEIN EXPRESSION IN HAIRY CELL LEUKEMIA: EFFECT OF PHORBOL ESTER TUMOR PROMOTERS ON SURFACE GLYCOPROTEINS, CELL MORPHOLOGY, AND ADHERENCE.

Lockney MW, Golomb HM, Dawson G
(c/o Dawson) Dept. Pediatrics, Joseph P. Kennedy Jr. Mental Retardation Res. Center, Pritzker Sch. Medicine, Univ. Chicago, Chicago, IL, 60637
Cancer Res; 42(9):3724-3728 1982

Hairy cell leukemia cells from eight different patients exhibited a characteristic cell surface glycoprotein pattern when labeled by the neuraminidase-galactose oxidase-NaB3H4 procedure. The diffuse high-mol-wt glycoprotein band (Mr 230,000 to 300,000) was not seen in other leukemic cell types and may represent a specific hairy cell leukemia antigen. Hairy cell leukemia cells can be maintained as cell suspension cultures, but treatment with a variety of tumor-promoting phorbol esters caused the cells to adhere to plates, assume a fibroblastic elongated shape, and extend long processes. This dramatic morphological change was not associated with any change in surface glycoproteins. (Author abstract) (20 Refs)

5. STUDIES OF THE PLASMA MEMBRANE IN THE DIFFERENTIATION OF NORMAL AND MALIGNANT THYMOCYTES.

Casteel N
University of Health Sciences/The Chicago Medical School, Chicago, IL
Diss Abstr Int [B]; 42(10):4009-B 1982

The role of the plasma membrane in the differentiation and activation of thymocytes and how cyclic nucleotide-related enzymes are altered with the progress of leukemia was investigated. Enzyme levels of thymocytes of normal, preleukemic and leukemic mice were compared. Both virally-induced leukemia of AKR mice and chemically-induced leukemias of Swiss mice injected with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) were studied. While total AMPase activity appeared erratic, 5'-nucleotidase dropped significantly while alkaline phosphatase activity rose dramatically in the thymocytes of both leukemic models. Preleukemic thymocytes showed intermediate values. This is in contrast to normal developmental changes, where the activities of 5'-nucleotidase and alkaline phosphatase rise in parallel. Phosphodiesterase activity of the

thymocytes of these mice also became elevated with leukemia, confirming that the enzymic changes that would lead to lowered cAMP levels and increased proliferation, ie, lowered 5'-nucleotidase and elevated phosphodiesterase activities are controlling factors in the development of both virally-induced and chemically-induced leukemias. (no Refs)

6. CELL SURFACE MEMBRANE CHANGES DURING THE DIFFERENTIATION OF HUMAN PROGRANULOCYTIC LEUKEMIA HL-60 CELLS (MEETING ABSTRACT).

Gupta SK, Felsted RL, Glover CJ, Bachur NR, Fischkoff S, Gallagher RE
University of Maryland, Baltimore, MD, 21201
Fed Proc; 41(3):900 1982

The human promyelocytic leukemic cell line, HL-60, was induced to differentiate in vitro by treatment with dimethylsulfoxide and retinoic acid. Cell surface membrane proteins and glycoproteins were labeled with I-125 by the methods of lactoperoxidase/hydrogen peroxide or Iodo-gen and analyzed by two-dimensional polyacrylamide gel electrophoresis and autoradiography. A minimum of twelve cell surface proteins were unchanged, three proteins (MW 95,000; 87,000; and 77,000) were lost and seven new components (MW 280,000; 250,000; 162,000; 143,000; 58,000; 56,000; and 50,000) appeared during cell differentiation. Both chemical inducers gave similar results. The major labeled cell surface protein (MW 95,000) lost during cell differentiation correlated with the loss of cell transferrin binding and was identified as the transferrin receptor by affinity absorption of extracts of I-125 surface membrane-labeled cells with transferrin-Sepharose beads. This affinity purified component had molecular weights of 200,000 and 95,000 daltons under non-reducing and reducing conditions, respectively, confirming its dimer structure. Two-dimensional electrophoresis of cell surface membrane labeled normal granulocytes confirmed the absence of the transferrin receptor and the presence of several of the major new cell surface proteins identified during HL-60 maturation. (no Refs)

7. THE MODIFICATION OF GLYCOPROTEIN BIOSYNTHESIS AS A POTENTIAL BASIS FOR CANCER CHEMOTHERAPY.

Morin MJ
State University of New York at Buffalo, Buffalo, NY
Diss Abstr Int [B]; 42(9):3625-B 1982

The biochemical and therapeutic effects of the sugar analogue 6-deoxy-6-fluoro-D-galactose (6-F gal) and the antibiotic tunicamycin (TM) were examined to establish the extent to which modification in glycoprotein synthesis were required for the expression of potentially useful alterations in tumor cell characteristics. Exposure of L1210 leukemic cells to non-growth inhibitory doses of 6-F gal apparently induced only subtle modifications in glycoproteins, the most prominent of which was a dose-dependent and time-dependent decrease in the activity of cell surface sialyltransferase. Exposure of L1210 leukemic cells to TM, which inhibited the first step in the synthesis of asparagine-linked glycoproteins, resulted in less subtle modifications in tumor cell behavior. Specific alterations in plasma membrane components and in tumor cell ultrastructure, including distentions in the endoplasmic reticulum and nuclear membrane, were apparent in cells treated with doses of the antibiotic at which glycosylation processes were selectively inhibited. Examination of the effects of the exposure of L1210 ascites to TM in vitro on the subsequent transplantability of these cells in vivo revealed a dose-dependent increase in the survival of normal mice without a significant decrease in the tumorigenicity of the same cells implanted into immunologically compromised mice. The demonstration of resistance to further challenge with a lethal inoculum of tumor cells among mice which survived the initial exposure to TM-treated cells gave further evidence of an immunological component to the dose-dependent increase in host survival. Attempts to invoke a similar response in tumor-bearing mice with a direct administration of TM were limited by the predominant hepatotoxicity of the antibiotic. The toxicity of TM in mice could be diminished with a prior infusion of D-mannose and a combination of the two resulted in enhanced inhibition of L1210 leukemic cell growth in vitro and gave some evidence of a minor increase in the survival times of tumor bearing mice. (no Refs)

8. NONCYTOLYTIC EXTRACTION OF MURINE TUMOR-SPECIFIC TRANSPLANTATION ANTIGENS WITH THE NONIONIC DETERGENT OCTYL-BETA-D-GLUCOPYRANOSIDE.

LeGrue SJ, Macek CM, Kahan BD
(c/o LeGrue) Dept. Surgery, Univ. Texas Health Science Center, Houston, TX, 77030
JNCI; 69(1):131-136 1982

The nonionic detergent octyl-beta-D-glucopyranoside (C3Glu) was used to extract immunogenic tumor-specific transplantation antigen (TSTA) from intact cells and purified plasma membranes of the 3-methylcholanthrene-induced fibrosarcoma MCA-F. Pretreatment of syngeneic C3H/HeJ mice with 100 ug of C8Glu extracts induced specific immunoprotection such that mice resisted the outgrowth of MCA-F but not the antigenically distinct tumors MCA-D or MCA-2A. Incubation of intact cells with 7 mM (0.2%) C8Glu for 30 minutes at 23 C was judged to be noncytolytic because extracted cells excluded trypan blue. Preparative isoelectric focusing partially purified the MCA-F-specific antigen from crude C8Glu extracts into the pH 6.5-6.8 region of the gradient. Electrofocusing yielded 60% of the applied antigen activity and a fourfold to fivefold increase in specific activity. In addition, immunoprospective activity was obtained in 2% C8Glu extract of MCA-F plasma membranes, confirming the membrane localization of the MCA-F TSTA. Three properties of C8Glu rendered it an attractive agent for the preparation of cell surface proteins: a nonionic character, large critical micellar concentration, and its capacity to extract antigens without complete membrane solubilization or cell disruption. (Author abstract) (30 Refs)

9. FURTHER STUDIES ON THE RELATIONSHIP BETWEEN ALLOTRANSPLANTABILITY AND THE PRESENCE OF THE CELL SURFACE GLYCOPROTEIN EPIGLYCANIN IN THE TA3-MM MOUSE MAMMARY CARCINOMA ASCITES CELL.

Miller SC, Codington JF, Klein G
(c/o Codington) Lab. Carbohydrate Res., Dept. Biological Chemistry, Harvard Medical Sch., Massachusetts General Hosp., Boston, MA, 02114
JNCI; 68(6):981-988 1982

The loss of strain specificity in the TA-St mammary carcinoma ascites cell during passage in ascites form in diseased syngeneic strain A mice was confirmed by the observation that TA3-MM/2 ascites cells were capable of progressive growth in seven foreign mouse strains. Support for the hypothesis that allotransplantability in the TA3-MM lines may be due to masking of cell surface histocompatibility H-2 7a 1 antigens by large glycoprotein (epiglycanin) molecules was obtained from the

finding that the capacity of the TA3-MM/2 ascites cell to absorb anti-H-2 7a 1 antibody was several times less than that of the parent TA3-St ascites cell, although it was greater than the capacity of the non-strain-specific TA3-Ha line. Further support for the location of epiglycanin molecules at the cell surface was obtained by transmission electron-microscopic observation of a high concentration of filamentous structures, usually 20-40 nm long, but often extending to 200-300 nm length at the TA3-MM/2 cell surface. Similar structures were also observed at the TA3-Ha cell surface but were not observed at the surface of the TA3-St ascites cell, a cell known to contain no detectable epiglycanin. Epiglycanin molecules, obtained by two different methods from TA3-MM/1, TA3-MM/2, and TA3-Ha ascites cells, were shown to be similar with respect to their capacities to inhibit the binding of 125I-labeled epiglycanin to its antibody, induced in the rabbit by TA3-Ha ascites cells in a radioimmunoassay. (Author abstract) (21 Refs)

10. PURIFICATION AND BIOLOGIC CHARACTERIZATION OF A MAJOR EPSTEIN BARR VIRUS-INDUCED MEMBRANE GLYCOPROTEIN.

Qualtiere LF, Chase R, Pearson GR
Dept. Microbiology, Univ. Saskatchewan, Saskatoon, Saskatchewan, S74 0W0, Canada
J Immunol; 129(2):814-818 1982

The Epstein Barr virus membrane antigen (MA) complex is composed of three major high mw glycoproteins designated gp 300/350, gp 200/250, and gp 85/90. In the experiments reported in this paper, the gp 300/350 glycoprotein was purified from MA-positive extracts prepared from the B-95-8 cell line. This was accomplished by tandem combination of ion-exchange and lectin chromatography. It was routinely possible to purify this glycoprotein 400-600-fold by this procedure. Sera from animals immunized with gp 300/350 purified by this approach neutralized EBV infectivity and mediated antibody-dependent cellular cytotoxicity (ADCC) demonstrating that both of these important antigenic determinants were expressed on this glycoprotein. The presence of an ADCC determinant on this molecule was further substantiated by the finding that monoclonal antibody to gp 300/350 blocked ADCC mediated by a human ADCC antibody-positive reference serum. (Author abstract) (27 Refs)

11. SURFACE ANTIGEN EXPRESSED IN HEMATOPOIETIC CELLS DERIVED FROM FV-4 MOUSE STRAINS.

Yoshikura H, Odaka T
Dept. Microbiology, Univ. Tokyo, P. O. Box Hongo, Tokyo 113, Japan
JNCI; 68(6):1005-1009 1982

Fv-4 locus controls the susceptibility of mice to ecotropic murine leukemia virus. Antiserum against Fv-4r/s mice with BALB/c background was raised in inbred BALB/c mice. In the complement-dependent cytotoxicity test, the antiserum killed the hematopoietic cells derived from Fv-4r/- mice but not those from Fv-4s/s mice, and the genetic cross experiments located the locus controlling the expression of the target molecule of the antibody in the close vicinity of Fv-4. In addition, the antigenic expression of the exogenously infecting MuLV suppressed the expression of the target molecule of the antiserum. The expressions of these two antigens appear to compete with each other on the cell surface. (Author abstract) (21 Refs)

12. IDENTIFICATION OF AN ONCOFETAL ANTIGEN (GP90) ON MURINE B16 MELANOMA CELLS.

Nishio C, Ishii Y, Ishii K, Kikuchi K
(c/o Ishii) Dept. Pathology, Sapporo Medical Coll., S1 W17, Sapporo 060, Japan
Eur J Cancer Clin Oncol; 18(6):579-588 1982

A xenoantiserum to murine B16 melanoma cells was developed by immunizing rabbits with cultured B16 melanoma cells. This antiserum could, after extensive absorption with normal C57BL/6 mouse tissues, react with syngeneic (B16) and allogeneic (HP) melanomas as well as with other murine neoplasms, including syngeneic 75S adenocarcinoma, allogeneic myeloma and leukemic T cell lines. The antiserum also cross-reacted with syngeneic fetal fibroblasts and with an allogeneic fetal fibroblast cell line (SC-1) either uninfected or infected with murine leukemia virus (MuLV). Immunoprecipitated material from B16 melanoma cell-surface glycoproteins that had been labeled with [125I] by lactoperoxidase and purified by a Lentil lectin column was analyzed by one-dimensional SDS- and two-dimensional polyacrylamide gel electrophoresis, which disclosed an acidic glycoprotein with a molecular weight (mol wt) of 90 K daltons. Absorption studies suggested that the 90 K mol wt glycoprotein represented the oncofetal moiety expressed in murine melanoma, carcinoma and fetal tissues. When the amount of this antigen in developing C57BL/6 mouse fetuses was measured by absorption assays, we found that it was expressed strongly in those fetuses just before birth. Binding and absorption studies demonstrated that the 90 K mol wt glycoprotein, while being expressed on a variety of fetal and neoplastic cells in mice, did not exist at detectable levels in normal tissues of adult C57BL/6 mice, including tissues of the thymus, lymph node, spleen, liver, brain, lung and kidney. (Author abstract) (26 Refs)

13. EXPRESSION ON CULTURED HUMAN TUMOUR CELLS OF PLACENTAL TROPHOBLAST MEMBRANE ANTIGENS AND PLACENTAL ALKALINE PHOSPHATASE DEFINED BY MONOCLONAL ANTIBODIES.

McLaughlin PJ, Cheng MH, Slade MB, Johnson PM
Dept. Immunology, Univ. Liverpool, P. O. Box 147, Liverpool L69 3BX, England
Int J Cancer; 30(1):21-26 1982

The cellular reactivity of six monoclonal antibodies (McAbs) produced to isolated human placental syncytiotrophoblast microvillous plasma membranes has been examined using a variety of normal and malignant cell types. Two McAbs reacted with antigenic determinants common to most normal human cells. Two other McAbs (H310 and H316) reacted predominantly with normal placental trophoblast and with lymphocytic cells, as well as with most transformed or neoplastic cultured cell lines. Two further McAbs (H315 and H317) identified fetal differentiation antigens expressed only on the membranes of normal placental trophoblast and of certain tumor cell lines. H317 has been shown to be specific for the heat-stable L-phenylalanine-inhibitable placental-type alkaline phosphatase isoenzyme. These latter McAbs (H315 and H317) may prove useful in monitoring of some human cancers. (Author abstract) (36 Refs)

14. PURIFIED MEMBRANE FRACTIONS FROM MAMMARY TUMOR CELLS ELICIT BIOLOGICAL REACTIVITY IN IN VITRO CELL-MEDIATED IMMUNE REACTIONS.

Buessow SC, Caldwell K, Fletcher MA, Lopez DM
Dept. Microbiology, Univ. Miami Sch. Medicine, Miami, FL, 33101
Eur J Cancer Clin Oncol; 18(6):595-604 1982

Lithium chloride (LiCl) aggregation followed by centrifugation through a sucrose step gradient was used to obtain purified plasma membranes from two sublines of mouse mammary adenocarcinoma. These two tumor lines were chemically induced by treatment of a hyperplastic nodule with 7,12-dimethylbenzanthracene (DMBA). One line, D1-DMBA-3, has been found to be immunogenic to the host of origin, while the other, D1-DMBA-2, does not elicit specific tumor immunity as previously tested in in vivo and in vitro immune reactions. The purified membrane fractions were assayed for protein, DNA and sialic acid content as well as enzymatic markers of membrane purity. When tested in blastogenesis and cytotoxicity reactions, membrane-containing fractions from the D1-DMBA-3 immunogenic tumor were found to be stimulatory to spleen cells of D1-DMBA-3 tumor bearers over a 10-fold protein concentration. Spleen cells from normal mice do not respond in these reactions to the various tumor fractions. No reactivity was observed when non-membrane-containing preparations were used as stimuli in the cell-mediated immune reactions. The specificity of these reactivities was further demonstrated by the lack of responses when fractions from the non-immunogenic D1-DMBA-2 tumor were tested in parallel in our in vitro assay. The data presented indicate that the procedure employed is useful for the isolation of membrane-associated, tumor-specific antigens which can be easily quantitated and still retain biological activity in in vitro tests of cell-mediated immunity. (Author abstract) (41 Refs)

15. EFFECTS OF ALKYLATING AGENTS ON CELL MEMBRANES OF MOUSE LEUKEMIC CELLS AND HUMAN ERYTHROCYTES (MEETING ABSTRACT).

Ankel E, Ring B, Holcenberg J
Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI, 53226
Fed Proc; 41(3):1477 1982

Treatment of human erythrocytes with 10(-3) M tris(2-chloroethyl)-amine (HN3) produces a protein profile after SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) with new high molecular weight bands. The drug does not change the cell membrane profile of L5178Y mouse leukemic cells nor does it effect the binding of Con A (concanavalin A). Uptake of aminoisobutyric acid (AIBA) into L5178Y cells is inhibited by 73% after 1 hr incubation with 10(-3) M HN3. Similar incubations with 10(-4) M and 10(-7) M HN3 increased uptake of AIBA between 14% and 150%. Mechlorethamine (HN2) at 10(-3) M for 1 hr produces negligible changes in the electrophoretic pattern of human erythrocytes or L5178Y cell. One hr incubations of L5178Y cells with varying drug-concentrations produced a 60% inhibition of AIBA uptake at 10(-3) M but increased uptake at the lower concentrations. The alkylating agent Trenimone at 10(-4) M drug for 3 minutes caused a twofold increase in subsequent uptake of AIBA whereas 60 minutes drug contact decreased AIBA uptake by 95%. The effect on the electrophoretic profile of cell membranes of the treated cells was negligible. The results suggest alkylation of erythrocyte membranes by HN3 and the possibility of membrane damage after short time incubation with Trenimone leading to increased uptake in AIBA. (no Refs)

16. O-GLYCOSYLATION OF THE MAJOR GLYCOPROTEIN OF THE MAT-B1 SUBLINE OF THE 13762 RAT ASCITES MAMMARY ADENOCARCINOMA (MEETING ABSTRACT).

Rockley NL, Melcher UK, Carraway KL
Department of Biochemistry, Oklahoma State University, Stillwater, OK, 74078
Fed Proc; 41(3):665 1982

To determine the subcellular site of O-glycosylation of proteins, we have examined the synthesis of ASGP-1, the predominant glycoprotein of 13762 mammary ascites tumor cells. The precursor of ASGP-1 (pre ASGP-1) can be isolated from galactose- or leucine-labeled cells by its association with peanut agglutinin (PNA)-bound agarose and elution by galactose. Pre-ASGP-1 has a lower density and a smaller molecular weight than ASGP-1. The processing of pre-ASGP-1 to ASGP-1 was demonstrated by pulse-chase analysis and gel filtration. The kinetics of incorporation of leucine into a PNA-binding component showed no lag, suggesting that glycosylation occurs concomitantly with protein synthesis. Polysomes obtained from uracil-labelled cells bound to PNA-agarose, permitting isolation of a t-RNA-associated glycoprotein. It has approximately the same elution weight as galactose-labeled pre-ASGP-1 on Sepharose CL-2B. These results suggest that the rough endoplasmic reticulum is the site of galactose attachment. (no Refs)

17. RELATIONSHIP BETWEEN XENOTRANSPLANTABILITY AND CELL SURFACE PROPERTIES OF ASCITES SUBLINES OF A RAT MAMMARY ADENOCARCINOMA.

Howard SC, Hull SR, Huggins JW, Carraway CA, Carraway KL
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JNCI; 69(1):33-40 1982

Cell surface properties of several ascites sublines of the 13762 rat mammary adenocarcinoma were compared in an effort to understand factors important to the xenotransplantability of these tumors into C57BL/6J mice. MAT-C, MAT-C1, and MAT-cMR6-S ascites sublines were xenotransplantable; MAT-B1, Ma-C2, and MAT-MR2-S were not. All of these sublines contained a large mucin-type sialoglycoprotein (ASGP-1) as a major cell surface component. MAT-B1 and MAT-cMR6-S ASGP-1 molecules were sulfated, but MAT-C1 and MAT-MR2-S incorporated little sulfate. The major oligosaccharide of MAT-C1 ASGP-1, a disialo-oligosaccharide, was present in very low amounts in the other sublines. ASGP-1 was detected readily in the plasma of animals with MAT-C1 or MAT-MR2-S tumors, but it was not detected readily in animals with MAT-B1 or MAT-cMR6-S tumors. Thus none of these significant features of ASGP-1 that differed among the sublines correlated with xenotransplantation. However, both MAT-C1 and MAT-cMR6-S sublines had branched cell surface microvilli. Moreover, MAT-C grown in mice after xenotransplantation had extensive, branched microvilli, even through only about 10% of the population of the cells grown in the rat had them. These results suggest that the branched microvilli may provide a protective mechanism, possibly by acting as a barrier to the approach of cytotoxic cells. (Author abstract) (20 Refs)

18. ALTERED GLYCOSYLATION OF CHORIOGONADOTROPINS FROM JEG-3 CELL CULTURE (MEETING ABSTRACT).

Kellen JA, Malkin DG, Chan AW, Malkin A
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Proc Am Assoc Cancer Res; 23:928 1982

Plant lectins are useful probes for studying protein glycosylation, and can be used to indicate chemical alterations in cell surface architecture and products from malignant cells. The JEG-3 model used in this study is derived from a human trophoblastic tumor and is known to actively secrete choriogonadotropins (CG) in cell culture. We studied binding of the secreted CG to Concanavalin A (Con A) and Ricinus conjugated Sepharose 4B columns, in comparison with the product of normal 3rd trimester placental cells and after a pulse of gonadoliberin (LHRF, 1 ug/ml), dbCAMP (1mM), Con A (100 ug/ml) and Wheat Germ lectin (100 ug/ml), respectively. CG was determined by radioimmunoassay (RIA) using an antibody to the beta subunit. Our results indicate that while the CG released by normal trophoblast cells into the culture medium is 90% Con A-bound, up to 40% of CG from JEG-3 is not bound to this lectin; however, 25% of this 'free' fraction binds to Ricinus and is therefore glycosylated, containing D-Gal moieties. Exposure of JEG-3 cultures to LHRF, Con A, and dbCAMP increased CG secretion, but did not alter the proportions of CG binding to these lectins. Wheat Germ agglutinin inhibited cell growth. We conclude that malignant cells of trophoblastic origin synthesize a substantial part of CG with an altered pattern of glycosylation. This pattern is characteristic and constant, it does not change with increase or decrease of CG synthesis in the cell culture nor with numerous passages. (1 Ref)

19. EFFECT OF CELLULAR FATTY ACID (FA) ALTERATION ON HYPERTHERMIC SENSITIVITY OF L1210 MURINE LEUKEMIA CELLS (MEETING ABSTRACT).

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Proc Am Assoc Cancer Res; 23:120 1982

Previous studies have suggested a correlation between the FA composition of bacterial cells and their thermosensitivity. To determine if such a correlation also exists in mammalian tumor cells, we compared the thermosensitivity of L1210 cells after altering their FA composition. Cells growing in RPMI 1640 with 5% fetal calf serum were modified with respect to their FA composition by supplementing the media with either 40 uM docosahexanoic acid (22:6) or oleic acid (18:1). Cells grown in 22:6 supplemented media were more sensitive and cells grown in 18:1 supplemented media less sensitive to a 42C exposure than cells grown in unsupplemented media. These contrasting effects on thermosensitivity increased as the concentration of supplemented FA increased from 0-40 uM and were apparent at all temperatures from 41-44C. When compared to cells grown in unsupplemented media, those grown in 22:6 supplemented media contained 300% more polyenoic and 70% less monoenoic FA in their cell phospholipids while those grown in 18:1 supplemented media contained 37% less polyenoic and 17% more monoenoic FA. Similar changes were noted in neutral lipid FA composition while no changes were noted in cellular cholesterol or phospholipid content. Electron spin resonance studies demonstrated a significant increase in membrane fluidity when cells were grown in 22:6 supplemented media and a decrease when cells were grown in 18:1 supplemented media. These data demonstrate that modification of the cellular FA composition of a mammalian tumor cell dramatically affects its thermosensitivity. (no Refs)

MORPHOLOGICAL STUDIES

20. SCANNING ELECTRON MICROSCOPY IN CELL BIOLOGY AND MEDICINE.

Tanaka K, Fujita T
Dept. Anatomy, Tottori Univ. Sch. Medicine, Yonago, Japan
Excerpta Med Int Congr Ser (545): 499 pp., 1982.

Scanning electron microscopic (SEM) studies of cells and tissues are presented in order to encourage advancement in the field. A variety of biomedical fields is represented and numerous high level preparation techniques and unique applications of SEM are described. Cell surface and dynamics, cell internal structure, endocrine and exocrine glands, epithelia, nerves and muscles, reticular (lymphoid) tissues and blood, respiratory tract, sensory organs, and circulatory, urinary, and reproductive systems are represented. Areas of interest to cancer researchers include exocrine glands and the associated myoepithelial cells, the adenohypophysis, the pancreatic islet intercellular canaliculi system, basement membrane structure, brain ventricles, circumventricular organs, the pineal organ, vascular autonomic plexuses and skeletal neuromuscular junctions, guinea pig tenia coli, the reticuloendothelial system, lymph node, thymus, peri-rectal lymphoid tissue, human tonsil, hematologic applications of SEM, myocardial microvasculature, application of SEM during surgery, human laryngeal mucosa, cells lining the respiratory tract, kidney glomerular epithelial foot process loss, renal glomerulus, mammalian ovary (normal and pathologic), and effects of ionizing radiation on epithelium of the organ. (1251 Refs)

21. HUMAN MONOCYTE MEDIATED CYTOTOXICITY TO K-562 CELLS: A FREEZE-FRACTURE-, SCANNING- AND THIN SECTION ELECTRON MICROSCOPIC STUDY.

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Acta Pathol Microbiol Immunol Scand [C]; 90(3):137-145 1982

The structural features of 'natural' human monocyte-mediated cytotoxicity towards K-562 leukemia cells in vitro have been examined. Human monocytes bound K-562 cells firmly and mediated a slow cytolysis of the leukemia cells. Monocyte binding of target cells was shown to be trypsin sensitive. Freeze-fracture and thin section electron microscopy revealed that effector and target cells were separated by an irregular space larger than 20 nm. There was no evidence for the involvement of specialized membrane junctions or organelle transfer between monocytes and target cells. Scanning electron microscopy revealed that K-562 cells bound to monocytes progressively lost their microvilli. This process started in the membrane areas close to the effector-target cell interaction. The results suggest that binding of target cells by monocytes followed by action of short-range soluble cytotoxic mediators may be the mechanism for the monocyte-mediated cytotoxicity towards K-562 cells. (Author abstract) (36 Refs)

22. FREEZE-FRACTURE STUDY OF THE INTERCELLULAR JUNCTIONS IN BASAL CELL CARCINOMA (BCC) (MEETING ABSTRACT).

Gasparini G, Contini D, Torti A
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Ninth Annual Meeting of the Society for Cutaneous Ultrastructure Research, April 1-3, 1982, Leiden, The Netherlands. Society for Cutaneous Ultrastructure Research 71 pp., 1982.

In order to study carefully the membrane specializations existing between the cells of BCC we have used the freeze-fracture technique, which allows an investigation of the detailed structure of cell membranes. The investigation has been carried out in 5 cases of BCC of the nodular type, 3 cases of the cystic type, and 2 cases of the superficial scarring type. Tumor cells are united by a relatively high number of desmosomes. Gap junctions between keratinocytes are more frequent in BCC than in normal epidermis. Tight junctions appear as an extensive, belt-like network of ridges and grooves in BCC of the cystic type, while in the nodular and superficial scarring types they appear as more or less continuous short strands. Frequently tight and gap junctions coexist and it is possible to observe small gap junctions surrounded by short strands and having a bizarre structure. In conclusion, gap junctions, which are rare in squamous cell carcinomas, are well represented in BCC and this fact might be related to a higher degree of cell growth control. The high number and extension of tight junctions in BCC of the cystic type may be associated with the presence of cavities inside of the tumor. Finally, the coexistence of tight and gap junctions may represent a good model for study of the relationship between these two types of junctions and of the membrane associated particles by which they are formed. (no Refs)

23. LYSOSOMOPHAGY IN CULTURED MACROPHAGES TREATED WITH THE ANTIMICROTUBULAR DRUG NOCODAZOLE.

Thyberg J, Blomgren K, Hellgren D, Hedin U
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Eur J Cell Biol; 27(2):279-288 1982

Thioglycollate-elicited mouse peritoneal macrophages were cultivated in vitro and treated with the antimicrotubular drug nocodazole. The drug produced a structural reorganization of the cells, inhibited endocytosis of horseradish peroxidase and stimulated extracellular secretion of the lysosomal enzymes beta-glucuronidase and beta-N-acetylglucosaminidase. The structural effects included formation of large bundles of 10 nm diameter filaments and dispersion of the dictyosomes of the Golgi complex from a circumscribed juxtanuclear region throughout the cytoplasm. There appeared an increased number of autophagic vacuoles of a type not described in detail before. They were formed by lysosomes enclosing other lysosomes. The rate of degradation of cellular protein was not clearly affected by nocodazole. A possible explanation could be that the material enclosed within the autophagic vacuoles made up only a small fraction of total cellular protein. Moreover, a large part of normal protein turnover in cultured cells occurs outside the lysosomes. Although lysosomal membranes evidently were broken down when the autophagic vacuoles were transformed into lysosomes, it remains to be shown to what extent the content of the entrapped lysosomes was turned over more rapidly than normal. It is suggested that the nocodazole-induced lysosomophagy represents a subtype of autophagy that serves the purpose to regulate the amount of lysosomes and especially of lysosomal membrane in connection with inhibited endocytosis. In the macrophage, a cell type specialized for endocytosis and intracellular degradation of exogenous material, lysosomophagy would be the counterpart of crinophagy in secretory cells. (41 Refs)

24. 5-NUCLEOTIDASE ACTIVITY IN LYMPHOID CELLS FROM CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AND LEUKEMIC NODULAR NON-HODGKIN'S LYMPHOMA (MEETING ABSTRACT).

Pangalis GA, Tsavaris NB, Laskaridou A, Kouzioka D, Fessas P
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Proc Am Soc Clin Oncol; 1:C-526 1982

We recently established a cytochemical method for the demonstration of 5-Nucleotidase (5N) on blood smears, after modifying the method of Wachstein and Meisel. The lymphocyte positivity was mainly localized in the cell membrane, with a few positive granules in the cytoplasm. For the interpretation of the reaction a scoring system of 0-3+ was introduced. Using this cytochemical method, we studied the 5N activity of the circulating cells from 22 patients with CLL and three patients with leukemic conversion of nodular poorly differentiated lymphocytic lymphoma (NPDL). All cases studied had a B immunologic phenotype. Ten normal individuals were used as controls. A significantly reduced 5N activity was found in the CLL lymphocytes, as compared to normal lymphocytes. In the three patients with leukemic conversion of NPDL the lymphoid cells expressed an increased 5N activity, with individual scores of 43, 41 and 28. Further studies are on the way in order to determine whether 5N may represent an enzyme marker of the B cell maturation, which may discriminate some subcategories of non-Hodgkin's lymphomas. (1 Ref)

MEMBRANE RECEPTOR/ BINDING SITES

25. ISOLATION AND CHARACTERIZATION OF PEANUT AGGLUTININ-RESISTANT EMBRYONAL CARCINOMA CELL-SURFACE VARIANTS.

Rosenstraus MJ, Hannis M, Kupatt LJ
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J Cell Physiol; 112(2):162-170 1982

Isolation and characterization of embryonal carcinoma cell lines resistant to the cytotoxic effect of peanut agglutinin (PNA) is described. Parental cells were the pluripotent embryonal carcinoma cell lines PSA-1 and a hypoxanthine phosphoribosyltransferase-deficient, ouabain-resistant subclone, OPST-42. PNA-resistant clones were isolated at a frequency of 4 x 10(-5) following mutagenesis. All eight clones tested remained resistant to PNA after growth in nonselective medium. Somatic cell hybrids between PNA-resistant cells and PNA-sensitive cells suggested that the PNA-resistance in the mutants was recessive. Binding assays demonstrated that resistant cells exhibited a two- to four-fold reduction in the total amount of PNA bound, compared with parental lines. All eight PNA-resistant cell lines formed well-differentiated teratocarcinomas in syngeneic mice and the tumors contained cells resistant to PNA. When incubated in suspension with endodermal cells, the PNA-resistant cells sorted out to form simple embryoid bodies. The PNA-resistant cells showed reduced expression of stage-specific embryonic antigen 1. Thus, embryonal carcinoma cells can form tumors, differentiate, and recognize differentiated cells in a sorting assay, even though they have a reduction

in expression of the embryo-specific cell surface structures that bind PNA and anti-stage-specific embryonic antigen-1 antibody. (42 Refs)

26. LECTIN-BINDING CHARACTERISTICS OF HUMAN NATURAL KILLER CELLS.

Vose BM, Blackledge G, Crowther D, Gallaher J
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Immunology; 46(3):619-627 1982

Human natural killer (NK) cells separated initially by density centrifugation of lymphocytes (E+) forming rosettes with sheep red blood cells (SRBC), were further fractionated on gradients of bovine serum albumin (BSA). Low density fractions contained effector cells which displayed high cytotoxicity against the NK-sensitive erythroleukemic cell line, K562. These low density cells, which expressed receptors for Fc and the monoclonal antibody OKM1, showed enhanced cytotoxicity when treated with lymphoblastoid interferon (IFN-alpha). They also showed an increased response to phytomitogen in comparison with unseparated cells or those recovered from high density fractions. Two lymphocyte subsets one of high and one of low lectin binding capacity were identified in the E+ populations by their reactivity with Lens culinaris agglutinin (LCA). High LCA binding was observed only in low density fractions and was associated with a marked enrichment of NK activity. This property was used to separate the NK active population in E+ cells by fluorescence-activated cell sorting (FACS). These data add a new dimension to the cell surface properties of human NK cells and suggest the presence of LCA-reactive glycoproteins which are either enriched in, or uniquely associated with, cells of the NK subset. The experiments indicate that lectins can serve as useful probes of lymphocyte function and provide the basis for effective cell sorting. (Author abstract) (26 Refs)

27. EVIDENCE THAT GALACTOSYLTRANSFERASE IS A SURFACE RECEPTOR FOR POLY(N)-ACETYLLACTOSAMINE GLYCOCONJUGATES ON EMBRYONAL CARCINOMA CELLS.

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J Biol Chem; 257(12):6871-6878 1982

Cell surface glycosyltransferases are thought to participate in a variety of cellular interactions, but their specific glycoside acceptors have received little attention. In this paper, poly(N)-acetyllactosamine glycoconjugates are shown to be the endogenous substrates for embryonal carcinoma (EC) cell surface galactosyltransferases. All controls have been performed to ensure a surface localization for the galactosyltransferase activity. The galactosylated product(s) is relatively insoluble in organic solvents, is larger than conventional glycopeptides following pronase digestion, and is highly sensitive to endo-beta-galactosidase degradation. Solubilized polylactosaminyl glycoconjugates serve as competitive exogenous acceptors for the surface galactosyltransferase. In addition, the endogenous galactosyl acceptor(s) reacts with antiserum directed against EC cell poly(N)-acetyllactosamines. Anti-EC antiserum inhibits galactosylation of endogenous acceptors, simultaneously stimulates galactosylation of an exogenous acceptor, and immunoprecipitates 74% of the reaction product. Differentiated EC cells no longer react with anti-EC antiserum and no longer show anti-EC antiserum effects on surface galactosyltransferase activity. Interestingly, forced galactosylation with UDPGal releases polylactosaminyl substrates from the cell surface. In the absence of UDPGal, glycoconjugate release is dramatically reduced. GDPMan cannot substitute for UDPGal, and a galactosyltransferase inhibitor prevents glycoside release from the cell surface. Thus, surface galactosyltransferase preferentially binds poly(N)-acetyllactosamine glycoconjugates and serves as at least one of their surface receptors on EC cells. (Author abstract) (27 Refs)

28. INTERFERON ENHANCES GUANYLATE CYCLASE ACTIVITY IN HUMAN LYMPHOMA CELLS.

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Biochem Biophys Res Commun; 107(1):150-156 1982

Treatment of the human Burkitt lymphoma derived cell line Daudi with electrophoretically pure human interferon alpha (IFA), which causes a rapid increase in the intracellular concentration of cyclic guanosine monophosphate (cGMP), enhances guanylate cyclase activity. The increase in guanylate cyclase activity in IFA-treated cells resembled that induced by 10 milliM sodium azide in in vitro systems. Neither partially purified nor electrophoretically pure IFA had any effect on guanylate cyclase activity in a cell-free system made from Daudi cells, however. These results strongly suggest that activation of guanylate cyclase in IFA-treated cells is mediated via interaction of interferon with its specific cell surface receptor. The increase in cGMP in IFA-treated cells was calcium-dependent and was abrogated by treatment with the cyclo-oxygenase inhibitors aspirin and indomethacin. (17 Refs)

29. STRUCTURE-ACTIVITY RELATIONSHIPS WITH A SERIES OF SYNTHETIC PHORBOL DIESTERS IN MOUSE EPIDERMAL CELLS IN CULTURE (MEETING ABSTRACT).

Berry DL
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Proc Am Assoc Cancer Res; 23:401 1982

Although inflammation and hyperplasia cannot be determined experimentally in vitro using primary mouse epidermal cells in culture, stimulation of cell proliferation, ODC (ornithine decarboxylase) and phospholipid biosynthesis can be readily determined. A series of 12-O-tetradecanoylphorbol-13-acetates (TPA), 12-O-decanoyl-phorbol-13-acetates and phorbol-12,13-didecanoates (PDD) with none, one, two, three and four double bonds in the long chain aliphatic esters were compared with TPA, PDD, 4-0MeTPA and RPA for stimulation of cell proliferation (as measured by DNA synthesis and autoradiography), stimulation of ODC, phospholipid biosynthesis, and competition with TPA for binding to the membrane binding site as measured by 3H-phorbol dibutyrate. The kinetics of DNA, ODC and phospholipid biosynthesis were similar for all compounds. However, the potencies of the compounds varied with respect to aliphatic chain length and degree of unsaturation. RPA, 12-O-delta2,4,6,8-tetradecatetraenoylphorbol-13 acetate, phorbol-12, 13-delta2,4,6,8-didecatetraenoate were more potent than TPA for DNA stimulation, one-fourth the potency for ODC stimulation, and equal to or better for both phospholipid and competition with TPA for binding. As the number of double bonds in the aliphatic esters decreased, the biological activity decreased such that TPA greater than PDD greater than RPA much greater than 4-OMe-TPA. Previous reports indicate that highly unsaturated phorbol diesters are non-promoting or incomplete promoters. (no Refs)

30. MECHANISMS OF ENHANCEMENT OF CELL TRANSFORMATION BY TUMOR PROMOTERS (MEETING ABSTRACT).

Hirakawa T, Kakunaga T, Sugimura T
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Proc Am Assoc Cancer Res; 23:394 1982

The object of this study is to know the relationship of the pleiotropic effects induced by tumor promoters to their enhancement of carcinogen-initiated cell transformation. The potency of promoters for enhancing the transformation was measured by exposing 3-methylcholanthrene-pretreated A31-1-1 cells (Balb/3T3 subclone) to promoters for 2 weeks and scoring the transformation 2 weeks thereafter. The relative potencies in enhancing transformation of dihydroteleocidin B (DHTB), teleocidin B (TV), phorbol-12,13-didecanoate (PDD), 12-O-tetradecanoyl phorbol-13-acetate (TPA), 4-O-methyl TPA, and phorbol were approximately 100:100:30:1: less than 0.01: less than 0.01. The early effects of promoters on the cells, ie, induction of DNA synthesis in Go-arrested cells, stimulation of arachidonic acid release from membrane, and inhibition of EGF (epidermal growth factor) binding to cell surface receptor, were equally induced by DHTB, TB, PDD, and TPA and were hardly induced by 4-O-methyl TPA and phorbol. When the metabolic inactivation of these promoters in the cultures was measured by analyzing the metabolites using thin layer chromatography or by assaying the biological activities remained, the relative resistancies of DHTB, TB, PDD, and TPA to the metabolic inactivation by the cells were in parallel with their transformation enhancing potencies. However, the accumulated amounts of the induced DNA synthesis and arachidonic acid release during 8 days of promoter exposure were not different between DHTB, PDD, and TPA. On the other hand, the accumulated inhibition of EGF binding by DHTB and PDD was markedly greater than by TPA. (no Refs)

31. ALTERATION OF CELL SURFACE RECEPTORS AND 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) ACTIVITY IN ISOLATED EPIDERMAL CELLS (MEETING ABSTRACT).

Perchellet JP, Kishore GS, Conrad EA, Boutwell RK
McArdle Laboratory for Cancer Research, Madison, WI, 53706
Proc Am Assoc Cancer Res; 23:377 1982

To further characterize the primary site of action of TPA at the plasma membrane level and study its relevance to the biological activity of TPA, isolated epidermal cells were incubated with a variety of compounds known to alter the ultrastructure of cell surface receptors, and the induction of ornithine decarboxylase (ODC) activity by TPA was investigated. Neuraminidase treatments that elicited a time- and dose-dependent release of sialic acid (up to 80% of the total sialic acid) did not affect the induction of ODC activity by TPA nor the uptake of [3H]TPA by the cells. In contrast, these neuraminidase treatments were able, in the same cell system, to decrease the uptake of diverse [125I]-labeled hormones and to enhance as much as the gangliosides the stimulatory effect of cholera toxin on basal and TPA-induced ODC activities. None of the other membrane-altering agents tested, including fucosidase, galactosidase, galactose oxidase, phospholipases A2 and C, and NaI0(4), could inhibit the induction of ODC activity by TPA. In addition, the alpha- and beta- adrenergic antagonists phentolamine and propranolol and the cholinergic antagonist atropine that competed effectively for the binding receptors of [3H]dihydro-alph-ergocryptine, [3H]dihydroalprenolol, and [14C]acetyl-choline, did not inhibit the uptake and the effect of TPA on epidermal ODC activity. (no Refs)

32. SOLUBILIZATION AND PURIFICATION OF THE PHORBOL ESTER TUMOR PROMOTER RECEPTOR FROM MOUSE BRAIN (MEETING ABSTRACT).

Ashendel CL, Boutwell RK
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Fed Proc; 41(3):492 1982

In order to understand the molecular mechanism of action of the potent mouse skin tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) and its interaction with the membrane bound TPA receptor, it was necessary to purify this protein from mouse brain, the tissue with the highest specific binding activity (25 pmol/mg particulate protein). Solubilization from the crude particulate preparation required detergent. 10 mg/ml solutions of mild detergents such as sodium cholate, 3-[(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), and Triton X-100 gave the highest yields of solubilized activity. Purification was accomplished by DEAE (diethylaminoethyl) cellulose column chromatography, gel filtration chromatography using Sephacryl S-300 and S-200, and hydroxylapatite column chromatography. All chromatography was done using buffers containing CHAPS at a concentration of 2.5 mg/ml. The specific binding activity of the purified protein was at least 50 times greater than the crude particulate protein. In order to assay the TPA binding by the cold acetone filtration method it was necessary to reconstitute the solubilized binding protein by dilution in the presence of soybean lipid and MgCl2. Purified, reconstituted protein had the same affinity for TPA as the crude particulate protein. Further characterization of the purified TPA receptor including preparation of antibody is being persued. (no Refs)

33. TROPHOBLASTIC CELLS OF THE HYDATIDIFORM MOLE CONTAIN A B1-SUBTYPE ADRENERGIC RECEPTOR.

Moore JJ, Workman L, Whitsett JA
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J Clin Endocrinol Metab; 55(2):341-346 1982

Both beta-1 and beta2-adrenergic receptors have been previously described in normal human placental homogenates; the cells upon whose surface membranes these receptors reside have not been identified. In order to show that a beta1-adrenergic receptor is present on trophoblastic cells, the cells which mediate maternal-fetal transport and produce placental hormones, beta-adrenergic receptors were demonstrated in membrane fractions of human hydatidiform mole. Microscopic sections of the mole samples used demonstrated edematous villi lined by trophoblastic cells with minimal nontrophoblastic (stromal or vascular) contamination compared with placenta. (-)-[3H]Dihydroalprenolol [(-)-[3H]DHA]binding to molar membranes was reversible and saturable to a single class of sites (Kd = 0.97 +-0.12 nM; n = 7; maximum binding capacity, 72.9 +-6.4 fmol/mg protein). (-)-[3H]DHA binding was associated with catecholamine-stimulated adenylate cyclase activity. Agonist competition for the molar beta-adrenergic receptor showed the order of potency to be (-)isoproterenol much greater than norepinephrine = epinephrine, characteristic of a beta1-adrenergic receptor subtype. Competition for (-)-[3H]DHA binding to trophoblastic membranes by the beta-adrenergic receptor subtype-specific agents metoprolol (beta1 selective) and zinterol (beta2 selective) was also characteristic of a homogenous subtype of beta1-adrenergic receptors. Because beta1-adrenergic receptors alone were seen on trophoblast cells, the beta2-adrenergic receptor in placenta must reside on nontrophoblastic elements (stromal or vascular endothelium).

No differences in beta-adrenergic receptor binding were seen related with ploidy (2 or 3 N), the presence or absence of a fetus, or the progression of the mole to choriocarcinoma. Two choriocarcinoma cell lines, BeWo and JEG-3, however, showed no specific (-)-[3H]DHA binding. Human trophoblast contains beta1-adrenergic receptors coupled to catecholamine-sensitive adenylate cyclase, supporting a role for catecholamines in the regulation of placental metabolism. (Author abstract) (26 Refs)

34. A CLONED CELL WITH NK FUNCTION RESEMBLES BASOPHILS BY ULTRASTRUCTURE AND EXPRESSES IGE RECEPTORS.

Galli SJ, Dvorak AM, Ishizaka T, Nabel G, Simonian HD, Cantor H, Dvorak HF
Dept. Pathology, Beth Israel Hosp., Boston, MA, 02215
Nature; 298(5871):288-290 1982

Cloned mouse cell line Ly1-2-NK-1+/11 was studied morphologically and evaluated whether it shared other properties of basophilic leukocytes. Analysis by direct binding of 125I-labeled monoclonal mouse IgE demonstrated that it expressed high-affinity plasma membrane receptors (FcepsilonR) specific for IgE antibody. The binding of 125I-IgE was specific: it was inhibited by unlabeled mouse IgE but neither by normal mouse IgG nor by rabbit IgG, even in 133-fold molar excess. Findings indicated that cells sharing morphological and biochemical features of basophilic granulocytes can mediate natural killer lysis. (37 Refs)

35. CHARACTERIZATION OF THE RECEPTORS FOR IGE ON MEMBRANES ISOLATED FROM RAT BASOPHILIC LEUKEMIA CELLS.

Isersky C, Rivera J, Triche TJ, Metzger H
Section Chemical Immunology, NIADDK, NIH, Bethesda, MD, 20205
Mol Immunol; 19(7):925-941 1982

Mast cells, basophils and a tumor analog, rat basophilic leukemia cells, have a surface receptor for immunoglobulin E (IgE). Membranes from such cells should be highly useful for the study of how the alpha- and beta-subunits of the receptor interact with the plasma membrane and function therein. Membranes were prepared from rat basophilic leukemia cells. The cells were reacted with radioiodinated IgE to saturate a fraction of their receptors and the radiolabel was used as a surface marker. When isolated from sucrose gradients, 40 +-10% yields of plasma membranes were obtained and these were typically purified 13-fold with regard to cellular proteins. Unoccupied receptors for IgE were almost all recovered (as assessed on membranes solubilized with detergent) as were surface-labeled proteins in general. The membrane-bound IgE appeared fully exposed as assessed with anti-IgE antibodies but surprisingly 70-80% of the unoccupied receptors present were unable to bind IgE when unsolubilized vesicles were tested. By electron microscopy the preparations appeared free of recognizable intracellular organelles. Though some of the membranes appeared multivesicular many showed large breaks in continuity. Assessment of permeability, carried out on dextran gradients, indicated that a significant number (greater than or equal to 50%) of the IgE-carrying membranes are not sealed. It is therefore most likely that both external and cytoplasmic aspects of the plasma membrane were exposed in many of the vesicles. Treatment of such membrane vesicles with trypsin or papain did not alter the apparent mol wt of the alpha-chain of the receptor or its binding activity for IgE. Additionally, when the vesicles were examined by enzyme-catalyzed iodination, no labeling of the receptor was observed under conditions where it is not labeled on intact cells (ie in the presence of IgE). These results suggest that either the alpha-chain of the receptor does not penetrate the membrane or that it cannot be modified by the particular probes we used. (Author abstract) (34 Refs)

36. ESTABLISHMENT OF GONADOTROPIN-RESPONSIVE MURINE LEYDIG TUMOR CELL LINE.

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J Cell Biol; 94(1):70-76 1982

Several clonal Leydig tumor cell lines have been established by adapting the transplantable Leydig tumor, M548OP, to culture. One of these cell lines, MLTC-1, has been characterized with regard to the gonadotropin-responsive adenylate cyclase system. The binding of 125I-labeled human chorionic gonadotropin (hCG) was blocked by excess unlabeled hCG and lutropin (LH) but not by follitropin, thyrotropin, or insulin, indicating the presence of specific receptors for hCG and LH. Based on the specific binding of hCG to isolated MLTC-1 membranes, the calculated dissociation constant was 1.0 +-0.2 x 10(-10) M. The receptors appeared identical to those from normal murine Leydig cells when analyzed by SDS PAGE and sucrose density gradient centrifugation. The molecular wt and sedimentation coefficient were 95,000 daltons and 8.5 S, respectively. MLTC-1 cells responded to hCG by accumulating cyclic AMP and producing progesterone. Cyclic AMP accumulation was time- and dose-dependent with a maximal accumulation occurring at approx 0.2 nM hCG. At saturating levels of hCG, cAMP levels reached a max by 30 min and then declined very slowly. Adenylate cyclase activity in membranes prepared from MLTC-1 cells was stimulated by hCG, LH, NaF, cholera toxin, and guanyl-5'-ylimidodiphosphate. Additionally, choleragen was found to ADP-ribosylate a membrane protein of 54,000 daltons. This protein resembles the proposed guanine nucleotide regulatory component in both size and choleragen-dependent reactivity. These data suggest that MLTC-1 cells possess a gonadotropin-responsive adenylate cyclase system consisting of a specific hormone receptor, a regulatory component, and a catalytic subunit. (Author abstract) (40 Refs)

37. PROLACTIN RECEPTORS IN CULTURED RAT MAMMARY TUMOR CELLS. UNMASKING OF CELL SURFACE RECEPTORS BY ENERGY DEPLETION.

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J Biol Chem; 257(12):6971-6977 1982

The level of specific binding of 125I-labeled ovine prolactin to primary cultures of 7,12-dimethylbenzanthracene-induced rat mammary tumors can be increased 2-to 20-fold by a variety of chemically distinct respiratory inhibitors or uncouplers of oxidative phosphorylation. Independent of the type of inhibitor used, binding increases when the cellular ATP level is decreased by at least 70%. This change is due to an apparent increase in the number of binding sites. Receptor affinity (Kd = 4 x 10(-10) M) is not affected. The binding of 125I-labeled epidermal growth factor to tumor cells is not similarly altered. Primary cultures of normal mammary gland from perphenazine-stimulated rats and tumor tissue slices also show increased prolactin binding in the presence of these inhibitors. In contrast, binding to slices from lactating mammary gland, liver, ovary, adrenal gland, or kidney is unaffected. The low level of

prolactin binding in untreated tumor cell cultures is not due to hormone degradation or rapid down-regulation of receptors. Electron microscope autoradiography indicates that in the presence of 2,4-dinitrophenol prolactin binding is limited to the cell surface. Sites that can be activated by energy depletion are also localized on the cell surface. Thus, prolactin receptors in proliferating normal and neoplastic rat mammary tissue exist in two forms on the cell surface, an active form that recognizes and binds prolactin and an inactive form that does not. When cellular ATP levels are normal, the inactive form predominates (2 to 20 inactive sites for each active site). When ATP levels are markedly decreased, receptors are converted to the active (binding) form. In target cells where prolactin is not trophic, inactive receptors are absent or energy depletion does not activate the binding sites. This pool of cryptic cell surface receptors may enable certain prolactin target tissues with the ability to rapidly change their level of available prolactin receptors and thus regulate their hormonal response. (Author abstract) (60 Refs)

TRANSPORT STUDIES

38. EXPRESSION IN FETAL HEPATOCYTES OF AMINO ACID TRANSPORT SYSTEMS SEEN IN HEPATOMA CELL LINES (MEETING ABSTRACT).

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Fed Proc; 41(3):1155 1982

A transport system for anionic amino acids for which cystine reacts as a transported analog has been observed independently in fetal human lung fibroblasts, and in the hepatoma cell HTC. We have now shown that as the pH is raised from 5 to 8, cystine becomes a transport substrate for the system in HTC at pH values about one unit lower than predicted by its titration curve. Skin fibroblasts derived from a 4-year old child, and also another heptoma cell line EC3, showed neither transport inhibition between cystine and glutamate nor any increase in cystine uptake with the above pH rise. Fetal rat hepatocytes in primary culture show like HTC both Na+-dependent and Na+-independent transport of anionic amino acids. The Na+-independent agency for which cystine and glutamate compete is highly similar to the corresponding system in HTC. Identity is supported by the analog inhibition pattern: inhibition by cystine, by glutamate, homocysteate, and other longer anionic amino acids, but scarcely by the short-chain analogs, aspartate, cysteate, or cysteinesulfinate. Support also comes from the highly similar rise in glutamate-inhibitable cystine uptake seen in the pH range 5.0 to 7.5. This component of uptake has been neither detected nor absolutely excluded in the differentiated hepatocyte of the adult rat. Tests continue for loss or gain of transport systems on differentiation or transformation. (no Refs)

39. GLYCINE UPTAKE BY CULTURED Y79 HUMAN RETINOBLASTOMA CELLS: EFFECT OF CHANGES IN MEMBRANE PHOSPHOLIPID FATTY ACID COMPOSITION (MEETING ABSTRACT).

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Department of Biochemistry, University of Iowa, Iowa City, IA, 52242
Fed Proc; 41(3):1213 1982

Neural tissue membranes contain large amounts of polyunsaturated fatty acids. To investigate the functional significance of this on membrane-related processes, glycine uptake by Y79 retinoblastoma cells cultured in medium supplemented with 10% fetal calf serum was studied. Glycine uptake is sodium dependent and occurs through two kinetically, distinguishable processes. The high-affinity system has a K'm of 34.2 umol and a V'max of 91.2 umol per minute mg/protein, whereas the low-affinity system has a K'm of 2.77 mmol and a V'max of 4.07 mmol per minute mg/protein. Cells grown in fetal calf serum have membrane phospholipids which are low in polyunsaturates, but they can be highly enriched in polyunsaturates by supplementing the culture medium with these fatty acids. This alters the kinetic parameters of high-affinity glycine uptake. Supplementation with the n-6 class of polyunsaturates, linoleate or arachidonate, or the n-3 class, linolenate or docosahexoenoate, causes a 3 to 4-fold increase in the V'max and K'm. By contrast enrichment with the n-9 monounsaturate, oleate, produces no change in either K'm or V'max. These data suggest that the high polyunsaturated fatty acid content of neural cell membranes influences the uptake of glycine, a putative neurotransmitter. Therefore, the high polyunsaturated content may impart special transport properties to neural cell membranes. (no Refs)

40. EFFECT OF CHEMOTHERAPEUTIC AGENTS ON THE PUTRESCINE TRANSPORT INTO R3327MAT-LU PROSTATE DERIVED TUMOR CELLS IN VITRO (MEETING ABSTRACT).

Heston WD, Fleischmann J, Bramlet G, Fair WR
Division of Urology, Washington University School of Medicine, St. Louis, MO, 63110
Proc Am Assoc Cancer Res; 23:886 1982

Cells which have had their synthetic polyamine synthetic pathway blocked have been reported to take up increased amounts of polyamines from the extracellular environment. As the chemotherapeutic agent methylgloxal bis-(guanylhydrazone) (methyl GAG) shares the polyamine transport mechanism, its activity may be enhanced by intracellular polyamine depletion. It is now possible to block polyamine synthesis with the irreversible inhibitor of ornithine decarboxylase, alpha-difluoromethylornithine (DFMO). We examined the effect of methyl GAG and of other chemotherapeutic agents on polyamine uptake into a DFMO pretreated rodent prostate tumor line, the R3327MAT-Lu. The followng agents were found not to have any effect on putrescine uptake even when tested at 100uM concentration: cis-platinum, doxorubicin, methotrexate, mitomycin-C, Carmustine, and 5 fluorouracil. (1 Ref)

41. INHIBITION OF URIDINE UPTAKE AND POTENTIATION OF PALA CYTOTOXICITY BY DIPYRIDAMOLE IN VITRO (MEETING ABSTRACT).

King ME, Howell SB
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Proc Am Assoc Cancer Res; 23:818 1982

Reutilization of serum uridine by neoplastic cells containing uridine kinase may reduce the antitumor effectiveness of N-(phosphonacetyl)-L-aspartate (PALA), an inhibitor of de novo pyrimidine synthesis. For this reason we have examined the effect of dipyridamole (DP), an inhibitor of membrane nucleoside transport, on uridine uptake in a variety of human tumor cell lines including HL60 promyelocytic leukemia, T-219 colon carcinoma, T-242 melanoma and T-24A astrocytoma. Cells in log phase were pre-incubated in varying concentrations of DP for 30 minutes and then exposed to 10 uM 3H-uridine for 30

minutes. We then examined the combined effects of PALA and DP on T-219 colon carcinoma in colony forming assay. These cells were plated in varying concentrations of PALA with and without 1 uM DP and colonies counted in 7-10 days. Plating median contained 10 uM uridine. The results suggest that DP, a drug readily available for use in man, substantially inhibits uridine uptake in neoplastic human cells, and at concentrations easily achieved in plasma, DP appears to potentiate PALA cytotoxicity by approximately 4-fold. (no Refs)

42. TRANSPORT OF ACIDIC NUCLEOSIDES IN HUMAN LYMPHOBLASTOID CELLS (MEETING ABSTRACT).

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Proc Am Assoc Cancer Res; 23:860 1982

It is known that 6-Azauridine (pKa 6.7) inhibits the proliferation of human lymphoblastoid (RPMI 6410) cells in culture (IC50 equals 0.7 uM) and its inhibitory effect can be blocked by the nucleoside transport inhibitor nitrobenzylthioinosine (NBTI), suggesting that azauridine enters these cells via this nucleoside transporter. Examination of the effect of azauridine on the initial rate of uridine transport in these cells, and in a uridine kinase deficient subline, has revealed that azauridine is a very poor inhibitor of uridine transport and that its inhibitory effect is strongly dependent on pH. The IC50 for inhibition of uridine (100 uM) transport was greater than 10 mM at pH 7.4 and was reduced to 4 mM at pH 6.0. In contrast, uridine transport in these cells (KT equals 150 uM) was not affected over the pH range of 5.5 to 7.6. Direct examination of the uptake of [5-3H]-6-azauridine confirmed that its transport is pH-dependent, and the midpoint of the pH curve was about 6.6. The KT for azauridine transport was approximately 5 mM at pH 6.0 and increased 2 to 5 fold at pH 7.4. Azauridine transport was inhibited by NBTI suggesting that it is a substrate for the nucleoside transporter. However, the high KT for its transport and the high IC50 for its inhibition of uridine transport indicate that it is a poor substrate for the transporter. 3-Deazauridine (pKa 6.5) inhibition of uridine (100 uM) transport was also pH-dependent with an IC50 of 4.5 and 0.8 mM at pH 7.4 and 6.0, respectively. More acidic nucleosides, 5-mercaptouridine (pKa 5.0) and orotidine (pKa 3.0), did not inhibit uridine transport even at pH 6. (1 Ref)

43. ADENYLATE CYCLASE COUPLED BETA2-ADRENERGIC RECEPTORS IN A SMOOTH MUSCLE TUMOR CELL LINE (MEETING ABSTRACT).

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Fed Proc; 41(3):1511 1982

The DDT1 MF-2 cell line was established by explantation from an androgen-estrogen induced leiomyosarcoma of the ductus deferens of a Syrian hamster. Since these cells morphologically resemble smooth muscle cells and contain alpha 1-adrenergic receptors, we chose to examine them for the presence of beta-adrenergic receptors. Binding of [3H]-dihydroalprenolol (DHA) to membranes of DDT1 cells grown in suspension culture was of high affinity, saturable, stereoselective and reversible. The dissociation constant for DHA was 0.8 nM with site number of 425 fmoles/mg protein. The order of agonist competition was isoproterenol greater than epinephrine greater than norepinephrine consistent with a beta 2-receptor interaction. Furthermore, Zinterol, beta 2-selective, was more effective than Metoprolol, beta 1-selective in competing for the DNA binding site. Also, the DDT1 cell beta 2-adrenergic receptor is functionally linked to adenylate cyclase as basal cyclic AMP accumulation (112pmol/10(6) cells/10') was stimulated by 10 uM isoproterenol (402pmol/10(6) cells/10'). Stimulation by isoproterenol was inhibited by 10uM propranolol (102pmol/10(6) cells/10'). In conclusion, the presence of both alpha 1-and beta 2-adrenergic receptors make the DDT1 cell an ideal model in which to study the actions of catecholamines on smooth muscle. (no Refs)

44. CATECHOLAMINE TRANSPORT AND ENERGY-LINKED FUNCTION OF CHROMAFFIN GRANULES ISOLATED FROM A HUMAN PHEOCHROMOCYTOMA.

Johnson RG, Carty SE, Scarpa A
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Biochim Biophys Acta; 716(3):366-376 1982

The structure and function of chromaffin granules of human pheochromocytoma was extensively investigated in a highly purified granule fraction obtained from a single specimen of human pheochromocytoma tissue. Pheochromocytoma chromaffin granules were analyzed for catecholamine, ATP, enkephalin, phospholipid, cytochrome and ion content. Using a variety of techniques it was found that the membrane of these granules is highly impermeable to Na+, K+, and H+, and that the intragranular pH was maintained at 5.1 irrespective of suspending media. The presence of MgATP induces a transmembrane potential (Deltapsi) across the membrane of these granules which is positive inside and which corresponds to 90 mV. both DeltapH and Deltapsi are coupled to biogenic amine accumulation into the granules in a process which is reserpine sensitive. These properties are compared with those of chromaffin granules isolated from normal tissue or from other animal species and are discussed in terms of possible explanation at a biochemical or subcellular level of the clinical manifestation of the pheochromocytoma. (Author abstract) (46 Refs)

45. A KINETIC ANALYSIS OF THE UPTAKE OF 5'-DEOXY-5-FLUOROURIDINE IN EHRLICH ASCITES TUMOR CELLS (MEETING ABSTRACT).

Enigbokan MA, Bowen D
Department of Pharmacology, Howard University, Washington, DC, 20059
Med Pediatr Oncol; 10(1):99 1982

The time course of uptake of a new cytotoxic agent 5'-deoxy-5-fluorouridine (5'dFUr) was determined at 37 degrees in unperturbed Ehrlich tumor cells. Short term uptake studies were determined by a rapid sampling technique which allows sampling of cell suspensions at intervals as short as 0.5 sec. The time course of 5'dFUr uptake is composed of an initial rapid uptake component which appears to represent a surface adsorption of the drug. This is followed by a slower uptake component of 5'dFUr which is constant for 20 sec and represents the unidirectional influx process. Transport across the cell membrane occurs by a carrier mechanism displaying an apparent Km of 38 uM and a Vmax of 1.52 nmoles/g dry wt/sec. Dipyridamole, a nucleoside transport inhibitor, inhibits 5'dFUr influx. Influx of 5'dFUr was inhibited by the homologous nucleosides, fluorouridine (FUr) and fluorodenoxyuridine (FUdR). Transstimulation of 3H-FUr, 3H-FUdR, and 3H-5'dFUr is demonstrated when cells are loaded with 5 mM non-labeled 5'dFUr. Hence, these data suggest the transport of 5'dFUr utilizes a mobile carrier system similar to fluoropyrimi-

dine nucleosides and, therefore, maybe a model substrate for the study of fluorinated nucleoside transport. (no Refs)

46. 5'-DEOXY-5-FLUOROURIDINE: A MODEL SUBSTRATE FOR THE STUDY OF PYRIMIDINE NUCLEOSIDE TRANSPORT (MEETING ABSTRACT).

Bowen D, Enigbokan MA, Diasio RB
Department of Pharmacology, Howard University, Washington, DC, 20059
Proc Am Assoc Cancer Res; 23:864 1982

It is known that 5'-Deoxy-5-Flurorouridine (5'dFUr) a new fluoropyrimidine (FP) nucleoside with antineoplastic activity was studied for its effects on the transport and metabolism of FP and natural occurring pyrimidine nucleosides in Ehrlich tumor cells. Transport of 5'dFUr occurs by a carrier mechanism with an apparent Km of 38uM and a Vmax of 1.52 nmoles/g dry wt/sec. Dipyridamole, a nucleoside transport inhibitor, inhibits 5'dFUr influx. Influx of 1uM 3H-fluorouridine (FUr) and 1uM 3H-fluorodeoxyuridine (FUdR) is inhibited 23% and 25%, respectively by 40uM 5'dFUr. 5'dFUr is a competitive inhibitor of 3H-FUdR influx. Trans-stimulation of 0.25uM 3H-FUr, 0.25 uM 3H-FUdR, and 0.25uM 3H-5-dFUr influx is demonstrated when cells are preloaded with 5mM nonlabeled 5'dFUr. The rate of 3H-FUr, 3H-FUdR, and 3H-5'dFUr influx is stimulated 22%, 54%, and 38%, respectively by 5'dFUr. The data suggest a mobile carrier system for 5-substituted FP nucleosides. This system also functions for thymidine (TdR) and deoxyuridine (UdR) influx. 5'dFUr stimulated 3H-TdR (0.25uM) and 3H-UdR (0.25uM) uptake into the soluble pool of Ehrlich cells 42% and 200%. 3H-TdR incorporation into DNA (TdRin) is stimulated 18%, but 3H-UdR incorporation into DNA (UdRin) is inhibited 99% by 5'dFUr. The inhibition of 3H-UdRin is related to the formation of fluorodeoxyuridylate (FdUMP) by 5'dFUr; whereas 3H-TdRin is unaffected by FdUMP but related to an increase in 3H-TdR transport. Hence these data suggest the transport of 5'dFUr utilizes a mobile carrier system similar to FP and natural occurring pyrimidine nucleosides, and therefore, may be a model substrate for the study of pyrimidine nucleoside transport. (no Refs)

47. VM-26 AND VP-16-213 INDUCED AUGMENTATION OF UPTAKE OF METHOTREXATE (MTX) AND ITS POLYGLUTAMYLATED DERIVATIVES IN EHRLICH ASCITES TUMOR CELLS IN VITRO (MEETING ABSTRACT).

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Department of Medicine, Medical College of Virginia, Richmond, VA, 23298
Proc Am Assoc Cancer Res; 23:847 1982

This evaluation of VM-26 and VP-16-213 effects on energy-dependent MTX transport and metabolism was prompted by previous reports of epipodophyllotoxin alterations of cellular energy metabolism. VM-26 (10uM) slows efflux of MTX which leads to an increase in the steady state level of exchangeable antifol. VM-26 stimulation of net MTX transport occurs within 5 min of exposure of cells to VM-26 (10-50uM); removal of extracellular (ec) VM-26 results in a rapid and complete reversal of this effect. Glucose partially reverses the stimulatory effect of VM-26 but to a lesser extent than glucose reversal of the vincristine-induced stimulation of net MTX transport. As VM-26 is increased from 5 to 50uM, the progressive rise in net MTX uptake observed is due not only to elevated exchangeable MTX but to an enlarged nonexchangeable pool of intracellular drug as well, a major portion of which is MTX polyglutamate (MTX-PG) derivatives. MTX-PG levels were further increased after one hour of incubation with 50uM VM-26 in MTX-free buffer indicating that VM-26 slows MTX efflux sufficiently to allow for significant additional MTX-PG formation even in the absence of ec MTX. VP-16-213 alos stimulates net MTX transport in a manner qualitatively similar to VM-26. However, 100uM VP-16-213 is required to yield the same quantitative stimulation as seen for 10uM VM-26. VP-16-213 also induced augmentation of MTX-PG levels. These results indicate that VM-26 and VP-16-213 enhance net cell accumulation of MTX and MTX-PG derivatives in the Ehrlich ascites tumor in vitro at concentrations utilized in vivo. (no Refs)

48. THE CELLULAR UPTAKE OF 4-HYDROPEROXYCYCLOPHOSPHAMIDE (MEETING ABSTRACT).

Redwood WR, Hilton J, Colvin M, Owens AH
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Proc Am Assoc Cancer Res; 23:664 1982

The alkylating agent 4-Hydroperoxycyclophosphamide (4-HC) is a 'preactivated' analogue of cyclophosphamide which exhibits in vitro the cytotoxic, immunoactive, and other biological properties of microsomally activated cyclophosphamide. This compound is currently used for in vitro studies of cyclophosphamide activity, such as tumor cell drug sensitivity studies. We have studied the uptake of 14C and 3H side chain labelled 4-HC into murine L1210 cells. The uptake of 4-HC by these cells is an active process which is rapid (maximum uptake within 90') and produces an intracellular drug concentration approx 4 fold the extracellular 4-HC concentration. The transport system has a Km of approximately 50uM and is inhibited by N-ethyl maleimide. 4-Hydroxycyclophosphamide was produced by the reduction of labelled 4-HC in order to study the transport of this cyclophosphamide active metabolite. The cellular transport of 4-hydroxycyclophosphamide appears to be similar to that of 4-HC. Our data indicates that 4-HC is transported into the cell intact. Inside the cell the 4-HC is rapidly converted to 4-hydroxycyclophosphamide, which through generation of phosphoramide mustard is responsible for the cytotoxicity of 4-HC. (no Refs)

49. SECRETION OF CISPLATIN AND EFFECTS OF PROBENECID: HUMAN AND RAT KIDNEY SLICE STUDIES (MEETING ABSTRACT).

Jacobs C, McGarrey K, Rich L, Weiner MW
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Proc Am Assoc Cancer Res; 23:493 1982

To investigate the nephrotoxicity of cisplatin (CP) we studied the renal handling of this drug by the human kidney and rat kidney slices. Our prior work suggested secretion of CP when platinum clearances (CPt) were compared to creatinine clearances (CCr). In the current study 6 patients with advanced cancer and normal renal function received CP (80-100mg/m(2)) by 24-hr infusion with a concurrent infusion of inulin (I) and para-aminohippurate (PAH). Multiple clearances of Cr, I, and PAH were obtained during the first 8 hrs. Total and free Pt (plasma ultrafiltrates) were determined by flameless atomic absorption spectrophotometry. Pt clearances were expressed as a fraction of CCR, CI and CPAH. Peak Pt levels occurred at 24 hrs. The data confirm secretion of CP or a metabolite by the human kidney. The fractional CPt was 40 +/- 3% of CPAH. To investigate the mechanism of secretion slice uptake studies were done in rat kidney. Rat slices concentrated

Pt at twice that in the medium, and this was blocked by metabolic inhibitors, demonstrating active transport. Ross and Gayle reported that probenecid, an inhibitor of secretion, reduced nephrotoxicity in rats. Two patients received probenecid-2gm/d prior to and during CP infusion resulting in no inhibition of secretion. Probenecid also failed to inhibit Pt uptake by rat kidney slices. Triethanolamine, an organic cation, did inhibit uptake. In conclusion CP or a metabolite is secreted by the human kidney, and in rat kidney slices active transport exists which may be blocked by an organic cation. (2 Refs)

50. TRANSPORT OF PHYSIOLOGICAL CONCENTRATIONS OF BENZO(A)PYRENE INTO HUMAN CELLS VIA A LIPOPROTEIN CARRIER (MEETING ABSTRACT).

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Department of Biological Sciences, North Texas State University Genetics Center, Denton, TX, 76203
Proc Am Assoc Cancer Res; 23:410 1982

Human serum lipoproteins were purified by size exclusion HPLC (high pressure liquid chromatography). Column effluent fractions were quantitated for capacity to bind [3H]-benzo(a)pyrene; binding was correlated with lipoprotein-cholesterol levels in the fractions. Efficiency of binding of benzo(a)pyrene to lipoproteins correlated directly to lipoprotein concentrations, and not to carcinogen concentration. Low density lipoproteins potentiated transfer of non-covalently bound [3H]-7,8-dihydrodiol-9,10-epoxybenzo(a)pyrene into non-mitogen-stimulated human lymphocytes held in vitro in RPMI 1640 for 24 hours without serum to deplete lipoproteins bound to the cell membrane. At high concentrations carcinogen entered the cells and was bound to lymphocyte DNA in the absence of low density lipoprotein, and without relation to time of exposure of the cell to carcinogen. When carcinogen at very low concentrations, 10 ng/ml, was introduced to cells non-covalently bound to low density lipoprotein, carcinogen binding to DNA increased as a function of time. Exposure to 10-fold increased concentrations of carcinogen in the absence of low density lipoprotein did not result in carcinogen bound to DNA. Adsorptive endocytosis of lipoproteins carrying non-covalently associated carcinogens represents a potential mechanism for intracellular transport of carcinogens available at physiological concentrations. (no Refs)

51. DOXORUBICIN (ADRIAMYCIN) TRANSPORT IN NUCLEATED AND NON-NUCLEATED CELLS SHOWS FEATURES OF A SIMPLE DIFFUSION TRANSPORT PROCESS (MEETING ABSTRACT).

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Clinical Pharmacology Branch, National Cancer Institute, Bethesda, MD, 20205
Proc Am Assoc Cancer Res; 23:14 1982

Previously published data indicated that doxorubicin (DOX) transport in human red cells is a simple Fickian diffusion transport process. The characteristics of DOX transport in the nucleated Ehrlich ascites tumor cells were investigated and compared with those of the non-nucleated human red cells. The Ehrlich ascites tumor cells (hyperdiploid strain) were maintained by weekly intraperitoneal transplantation in white female NMRI mice and harvested 8 days after transplantation. The apparent permeability coefficient in ascites cells and in red cells was of the same order of magnitude when calculated from the initial net uptake at 37 C and pH 7.3. DOX was strongly absorbed to the cell surface of ascites cells in contrast to the adsorption to red cells. The absorbed DOX was removed by washing the cells either with a DNA or a human albumin salt solution indicating the adsorption was reverisble to cell surface components in front of the rate limiting barrier. Cell membrane modifiers, 1-alcohols, local anesthetics, phloretin, HgCl2, parachloromercuribenzoate, affected DOX transport in ascites tumor cells and red cells in the same manner as these modifiers affected the transport of other lipophilic compounds. Ehrlich ascites tumor cells and human red blood cells appeared to represent two extremes with regard to DOX adsorption to cell surfaces but DOX transport through the rate limiting barrier of the cell membrane might be simple diffusion transport process of the uncharged DOX molecule in both cell types. (1 Ref)

52. TRANSMEMBRANE POTENTIAL CHANGE (TPC) OF SENSITIZED HUMAN LEUKOCYTES BINDING ORGAN-SPECIFIC CANCER NEOANTIGENS (OSN) (MEETING ABSTRACT).

Shenouda G, Thomson DM
The Montreal General Hospital Research Institute, Montreal H2G 1A4, Canada
Fed Proc; 41(3):311 1982

In this study we measured the TPC of leukocytes before and after exposure to extracts of the sensitizing or control cancer. The TPC was measured by the uptake of the permeant lipophilic cation [3H-Phenyl]Tetraphenylphosphonium bromide which equilibrates rapidly across biological membranes and the measurement of its distribution reflects the magnitude and direction of TPC. Leukocytes from early breast cancer patients when challenged with the breast OSN showed TPC. Leukocytes from patients with advanced breast cancer showed no TPC when challenged with breast OSN, unless the leukocytes were exposed before to PGE2 5 x 10(-6) M. The TPC was immunologically specific. The mechanism of the TPC was characterized. Monocytes armed with cytophilic antitumor antibody showed TPC when challenged with the sensitizing OSN, whereas T-lymphocytes did not. The TPC of leukocytes consisted of 2 reactions. There is TPC of the armed monocytes when they bind the OSN that occurred in the presence of ETYA or FPL 55712; and there is TPC induced in bystander cells by leukotrienes released from the monocytes when they bind OSN that could be blocked by ETYA or FPL 55712. TPC is an early event in stimulus-response coupling of human leukocytes reacting with their sensitizing cancer extracts. (no Refs)

53. TEMPERATURE DEPENDENCE AND EFFECT OF MEMBRANE LIPID ALTERATION ON MELPHALAN TRANSPORT IN L1210 MURINE LEUKEMIA CELLS.

Burns CP, Dudley DT
Dept. Medicine, Univ. Iowa Hospitals, Iowa City, IA, 52242
Biochem Pharmacol; 31(11):2116-2119 1982

The effects of temperature and lipid composition of cell phospholipids on the transport of melphalan (MP) were examined in L1210 lymphoblastic leukemia cells from male DBA/2 mice fed either sunflower seed oil or coconut oil diets. The transition temperature for MP transport by cells from mice fed the sunflower seed oil diet, which was rich in polyunsaturates, was 18.7 +-0.5 C. In contrast, the transition temperature was 22.5 +-1.2 C for cell from animals fed the coconut oil diet. The 3.8 C difference associated with the lipid alteration seems to reflect a lipid phase change. The lower transition temperature for MP transport agreed with the transition temperature for electron spin resonance studies, providing more evidence that the break in the Arrhenius plot for MP transport by the

L1210 cells is probably due to a lipid phase change. No differences in the maximal velocity or Michaelis constant were observed for MP transport in mice fed the sunflower seed oil or coconut oil diets. The results indicated that the discontinuity in the Arrhenius plot was the result of lipid phase transition and not of an interaction of the two carrier-mediated transport processes. (11 Refs)

MEMBRANE BIOGENESIS AND METABOLISM

54. MURINE B LYMPHOMAS: MODELS FOR IMMUNOGLOBIN EXPRESSION IN B CELL DEVELOPMENT.

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Diss Abstr Int [B]; 42(9):3582-B 1982

Individual clones of three B lymphoma cell lines (A20-1.11, X16C-4.3, K46-056) were found to synthesize two intracellular species of gamma 2a heavy chain which differ in apparent size by approx 6,000 daltons. The smaller gamma 2a heavy chain species is secreted by the cell and the larger gamma 2a heavy chain species is localized on the external cell membrane. Two species of mRNA, which apparently encode the two gamma 2a heavy chain species, were detected in the cytoplasm of the cells. It appears that the smaller (2.0 kb) mRNA species codes for the secreted gamma 2a heavy chain and the larger mRNA species (4.0 kb) codes for the membrane gamma 2a heavy chain. Fusion of the A20-1.11 or X16C-4.3 B lymphoma to myeloma cell lines results in hybrids expressing the more differentiated myeloma phenotype. That is, the expression of the secreted form of the lymphoma gamma 2a heavy chain is amplified as much as 150 fold while the expression of the membrane form of the lymphoma gamma 2a heavy chain polypeptide and corresponding mRNA is greatly diminished or abolished in the hybrid. Less than five percent of normal B lymphoid cells or plasma cell tumors express lambda light chains, and only rare tumors have been described that simultaneously express both lambda and kappa light chains in a single cell. However, three cloned B lymphoma cell lines (K46-056, B17-1.11, X16C-4.3) were found to express both lambda and kappa light chains. Different patterns of assembly of the light chains to the heavy chains were found. (no Refs)

55. A PHORBOL ESTER TUMOR PROMOTER INDUCES CHANGES IN THE EXPRESSION OF IMMUNOGLOBULINS AND DR ANTIGENS IN HUMAN LYMPHOBLASTOID CELLS.

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J Immunol; 129(2):539-544 1982

The present study indicates that 12-O-tetradecanoyl phorbol 13-acetate (TPA) can induce either increases or decreases in the relative expression of membrane-associated IgM and IgD as well as the expression of other markers of lymphocyte differentiation on human B lymphoblasts, depending on the clone studied. The changes in membrane Ig expression are apparent within 24 hr and are maximal at about 48 hr after the addition of the compound. In clone BL, TPA decreases the membrane IgM/IgD ratio as determined by the percentage of cells bearing these isotypes and by their densities on single cells. In clone MW-E, TPA has the opposite effect. Although these changes are followed by an increase in Ig secretion by MW-E cells, measured by immunofluorescence and the protein A plaque assay, There is no induction of Ig secretion in BL cells. In addition, TPA increases the expression in BL of an additional B lymphocyte marker, the D region-related (DR) antigens, while having the opposite effect in MW-E. In two sublines of Daudi, a membrane IgM-positive, IgD-negative, nonsecreting cell line, TPA treatment resulted in the expression of membrane IgD without the induction of Ig secretion. Thus, the TPA-induced changes in the IgM/IgD ratio and Dr antigen expression are inversely related and are not due to differential proliferation of subpopulations. Taken together, it appears that, depending on the particular clone of B lymphoblasts studied, TPA can induce or inhibit a coherent program of B lymphocyte maturation. (Author abstract) (52 Refs)

56. CYTOPATHIC ALTERATIONS INDUCED IN MOUSE NEUROBLASTOMA CELLS BY A TEMPERATURE-SENSITIVE MUTANT OF VESICULAR STOMATITIS VIRUS.

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Diss Abstr Int [B]; 42(7):2701-B 1982

Morphological alterations in mouse neuroblastoma cells were extensive following infection with temperature-sensitive (ts) mutant G31 of vesicular stomatitis virus (VSV) and incubation at 39 C. By 24 h post infection 80% of the mitochondria had degenerated becoming amorphous and losing physiological function, resulting in a decrease in cellular ATP. Intracytoplasmic vacuole formation also occurred and appeared to be independent of mitochondrial degeneration. Virus protein synthesis was required to produce these cytopathic effects but synthesis of a mature matrix and glycoprotein (G protein) was not. Another cytopathic effect in neuroblastoma cells during nonpermissive infection with ts G31 was cell fusion, with 90% of the cells having fused by 15 h post infection. This cell fusion, which required viral RNA and protein synthesis and could be blocked by adding anti-G protein IgG to infected cells, occurred only in nervous system-derived cells. The cell fusion also was inhibited by adding chloroquine to infected cells after virus uncoating had occurred. By indirect immunofluorescence the viral G protein could not be detected on the surface of infected and chloroquine-treated cells, but the G protein was present inside these cells. Removal of the chloroquine allowed the G protein to accumulate at the cell surface and cell fusion occurred. Tunicamycin, an inhibitor of glycosylation, could not prevent that accumulation. Therefore, the G protein was being synthesized and glycosylated in the presence of chloroquine, but was prevented from being expressed at the cell surface. (no Refs)

57. MODULATION OF HELA CELL PHOSPHOLIPID METABOLISM AND ORNITHINE DECARBOXYLASE ACTIVITY BY TUMOUR PROMOTERS: REGULATION OF RESPONSE TO PHORBOL-12,13-DIBUTYRATE BY RECEPTOR OCCUPANCY TIME.

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Biochem Biophys Res Commun; 106(4):1398-1404 1982

HeLa cell responses were measured, including the promoter-stimulation of phosphocholine release, [3H]choline incorpora-

tion into phospholipids, release of arachidonic acid, and the induction of ornithine decarboxylase (ODC) activity. The washing procedure of HeLa cells removed approx 96% of the [3H]phorbol-12,13-dibutyrate (PDBu) from cells pre-exposed to the ligand. The removal resulted in an essentially complete cessation of the release of radioactivity from cells pre-loaded with [3H]choline or [3H]arachidonic acid. A PDBu-stimulated incorporation of [3H]choline into phospholipids was still apparent after washing, but the magnitude of the response was considerably decreased. Washing cells which had been exposed to 12-O-tetradecanoylphorbol-13-acetate did not prevent the subsequent biological responses to the compound, presumably reflecting its higher affinity for the promoter binding site. Continuous exposure of cells to PDBu resulted in a peak of ODC activity at approx 5.5 hr, with a return to control values after 7 hr. Exposure to PDBu for 1.5 hr or 3 hr followed by washing did not lead to enzyme induction. Results indicated that continued receptor occupancy is required to maintain the early changes to membrane phospholipid metabolism induced by phorbol ester promoters. Prolonged receptor occupancy was also required for the promoter induction of ODC activity. (24 Refs)

58. REVERSIBLE ACQUISTION OF SARCOMA SURFACE ANTIGEN BY TRYPANOSOMA CRUZI (MEETING ABSTRACT).

Chess Q, Acosta AM, Sethi VK, Santos-Buch CA
Cornell University Medical College, New York, NY, 10021
Fed Proc; 41(3):483 1982

The ability of Trypanosoma cruzi, the obligate intracellular parasite and etiologic agent of Chagas' disease, to acquire host cell surface antigen was tested. WOS human sarcoma cell lines express a surface antigen (SSA) which is detected by an IgM anti-SSA with immune-adherence or immunofluorescence assays. When WOS monolayers were infected with T cruzi, the parasites produced in these monolayers acquired SSA. Indirect immunofluorescence showed IgM anti-SSA localized on the outer membrane of both intracellular (amastigote) and extracellular (trypomastigote) forms. When SSA-negative MNS fibroblast monolayers were infected, the parasites produced did not show SSA. Deletion of T cruzi-SSA could be accomplished by: 1) a single passage through SSA-negative MNS monolayers; or 2) multiple replications in SSA-negative cell-free medium. These experiments demonstrate reversible acquistion of SSA by T cruzi. This phenomenon, antigenic modification of parasite by host, may permit the parasite to 'masquerade as self' and thus escape immune destruction. It is also possible that such juxtaposition of host and parasite antigens ultimately induce certain 'autoimmune' reactions characteristic of chronic Chagas' disease. (no Refs)

CELL ADHESION

59. EFFECTS OF THE ANTICOAGULANTS, SODIUM WARFARIN AND SODIUM HEPARIN, ON RAT C6 GLIOMA CELLS IN VITRO.

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Diss Abstr Int [B]; 42(6):2327-B 1982

The rat C6 glioma cell line was selected as a model to test cytotoxic effects of the anticoagulants, sodium warfarin and sodium heparin, on brain tumor cells in vitro. Treatment of the cells with sodium warfarin during seven day growth experiments caused a dose-dependent inhibition of cell growth with decreased cellular adherence to the culture plates. Only at the highest dose, 10-2M, was cell viability diminished. Sodium heparin, even at doses as high as 630 units/plate, showed no inhibition of cell growth or cellular adherence and no decrease in cell viability. No changes in cellular protein levels were observed after treatment with 10-3M warfarin or 63 units of heparin. Three hour treatment with 10-3M warfarin yielded no biologically significant changes in 3H-thymidine and 14C-leucine uptake levels or incorporation into DNA or proteins, respectively. Twenty-four hour warfarin treatment caused a significant decrease in 3H-thymidine incorporation into DNA and a significant decrease in 14C-leucine incorporation into protein, when compared to control cells. Treatment of cells with 63 units of haparin did not have any effects on 3H-thymidine uptake or incorporation into DNA after 3 hour or 24 hour heparin incubations. Heparin also did not alter the synthesis of protein. Colony formation studies of interactions between the anticoagulant and selected anticancer agents gave the following results. Heparin is additive with busulfan, 5-fluorouracil, methotrexate, and vincristine. Heparin has a greater than additive effect with BCNU (carmustine), VM-26, and chlorambucil. Warfarin is less than additive with vincristine and VM-26. Warfarin is additive with 5-fluorouracil and busulfan, and warfarin has more than additive effects with BCNU, chlorambucil, and methotrexate. These results suggest that the anticoagulants deserve further study as possible adjuncts to glioma chemotherapy. (no Refs)

60. CODISTRIBUTION OF HEPARAN SULFATE PROTEOGLYCAN, LAMININ, AND FIBRONECTIN IN THE EXTRACELLULAR MATRIX OF NORMAL RAT KIDNEY CELLS AND THEIR COORDINATE ABSENCE IN TRANSFORMED CELLS.

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J Cell Biol; 94(1):28-35 1982

We used antibodies raised against both a heparan sulfate proteoglycan purified from a mouse sarcoma and a chondroitin sulfate proteoglycan purified forma rat yolk sac carcinoma to study the appearance and distribution of proteoglycans in cultured cells. Normal rat kidney cells displayed a fibrillar network of immunoreactive material at the cell surface when stained with antibodies to heparan sulfate proteoglycan, while virally transformed rat kidney cells lacked such a surface network. Antibodies to chondroitin sulfate proteoglycan revealed a punctate pattern on the surface of both cell types. The distribution of these two proteoglycans was compared to that of fibronectin by double-labeling immunofluorescent staining. The heparan sulfate proteoglycan was found to codistribute with fibronectin, and fibronectin and laminin gave coincidental stainings. The distribution of chondroitin sulfate proteoglycan was not coincidental with that of fibronectin. Distinct fibers containing fibronectin but lacking chondroitin sulfate proteoglycan were observed. When the transformed cells were cultured in the presence of sodium butyrate, their morphology changed and fibronectin, laminin, and heparan sulfate proteoglycan appeared at the cell surface in a pattern resembling that of normal cells. These results suggest that fibronectin, laminin and heparan sulfate proteoglycan may be complexed at the cell surface. The proteoglycan may play a central role in the assembly of such complexes since heparan sulfate has been

shown to interact with both fibronectin and laminin. (Author abstract) (43 Refs)

61. LAMININ INHIBITS ATTACHMENT OF NORMAL AND TRANSFORMED MURINE MACROPHAGES (MEETING ABSTRACT).

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Proc Am Assoc Cancer Res; 23:172 1982

Metastatic tumor cells must adhere to the capillary basement membrane of distant organs before extravasation. The importance of this step has prompted studies on the regulation of adhesive characteristics of neoplastic cells. Laminin, a basement membrane glycoprotein, has been shown to serve as an attachment factor for some metastatic tumor cells. In the present study the adhesive behavior of a highly metastatic murine tumor, M5076, was investigated in vitro in the presence and absence of purified exogenous laminin. The presence of laminin in adhesion assays stimulated the attachment of normal embryo fibroblasts, murine melanoma cells and fibrosarcoma cells to collagen IV and tissue culture plastic. In contrast laminin inhibited the attachment of M5076 reticulum sarcoma cells to these same substrates. This inhibitory effect appeared to be related to the macrophage origin of the M5076 tumor as indicated by the finding that the attachment of normal murine peritoneal exudate cells and WEHI-3, another macrophage tumor, was similarly inhibited. Furthermore, the effect was dose dependent, was reversible, and had no effect on the viability or functional capacity of the treated cells. This activity was a consequence of laminin binding to the substrates rather than to the cells, as demonstrated by separate preincubation studies. These results show that regulation of the adherence behavior of metastatic tumor cells is not universal but depends on the origin of the tumor. (no Refs)

62. CELL SURFACE ALTERATIONS ASSOCIATED WITH TRANSFORMATION AND TUMORIGENICITY IN HUMAN CELLS.

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Diss Abstr Int [B]; 42(9):3573-B 1982

The model system utilized for these studies consisted of a series of HeLa/fibroblast hybrids. Although these hybrids continued to express a transformed phenotype in culture, they were completely suppressed for tumorigenicity. From these suppressed hybrid populations, rare tumorigenic segregant subpopulations arose which had regained tumorigenic potential. The relationship between fibronectin expression and tumorigenicity was evaluated in these genetically similar nontumorigenic and tumorigenic hybrids. Quantitation of surface fibronectin expression by immunofluorescence and by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) of iodinated cell surface proteins demonstrated on correlation between the decreased level of fibronectin and tumorigenicity. The relationship between cytoskeletal organization and tumorigenicity was analyzed by immunofluorescence. Complex microtubule organization was found in all cells, independent of tumorigenicity. Although disorganized microfilaments were observed in tumorigenic hybrids, the organization of microfilaments was found to be related to cellular morphology and not tumorigenic potential. Double stain immunofluorescence demonstrated a close relationship between surface fibronectin and cytoplasmic microfilaments. Finally, the antigenic profiles of the hybrids were evaluated by utilizing antisera generated against either live nontumorigenic or tumorigenic hybrids. By immunofluorescence and immunoprecipitation analysis, no unique nontumorigenic hybrid antigens were identified. However, a single cell surface antigen of molecular weight 75,000 (75K protein) was identified that correlated exclusively with tumorigenicity. The 75K protein was not synthesized in fibroblasts or nontumorigenic hybrids and represented only 0.03% of the total cellular protein in HeLa and tumorigenic hybrids. Radiolabeling studies demonstrated its glycosylated nature and identified the 75K protein as a phosphoprotein. (no Refs)

63. PROSTAGLANDIN INHIBITION OF PMA INDUCED TUMOR CELL ADHERENCE (MEETING ABSTRACT).

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Department of Pathology, University of Michigan Medical School, Ann Arbor, MI, 48109
Fed Proc; 41(3):1078 1982

Tumor cell metastasis is thought to be initiated by the adherence of circulating tumor cells to endothelial cells and/or basement membrane. Several studies have suggested that the local generation of inflammatory mediators can alter tumor cell adhesiveness resulting in increased metastasis at sites of inflammation. Recent studies in our laboratory have demonstrated increased adherence of Walker Carcinosarcoma cells (WCC) to plastic plates, bovine corneal endotheial cells, and nylon fibers when stimulated with trypsinized fragments of C5, f Met-Leu-Phe, and phorbol myristate acetate (PMA). When WCC are pre-treated with PGE2 (10-5M) or 15-Methyl-PGE1 (10-5M) in the presence of theophylline (2.5 x 10-4M) there was 43.4 and 89.1% inhibition of PMA induced adherence to plastic plates. Thromboxane B2 and PGE2 did not significantly alter PMA induced adherence. WCC pretreated with histamine (10-4M), cholera toxin (10-6M), and dibutyryl cAMP (10-5M) showed similar inhibitory effects. These studies demonstrate that the anti-aggregatory prostaglandins 15-M-PGE1 and PGI2 can inhibit tumor cell adherence in vitro through their direct action on tumor cells. These effects may be mediated through alteration of intracelular cAMP levels. (no Refs)

64. FACTORS INFLUENCING THE SHEDDING OF SURFACE COMPONENTS OF EHRLICH CELLS (MEETING ABSTRACT).

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Department of Physiology, University of Texas Health Science Center, San Antonio, TX, 78284
Fed Proc; 41(3):755 1982

Ehrlich ascites tumor cells, when incubated in vitro, spontaneously shed cell surface or glycocalyx components into the medium. Exposure of cells to trypsin at low concentrations (1-5 ug/ml; 5-30 min at 37C) increases both the rate and amount of glycocalyx material released. The SDS (sodium dodecyl sulfate)-polyacrylamide gel electrophoretograms of the glycocalyx isolated from spontaneously shedding cells and trypsinized cells are essentially identical. Exposure of cells to 3H2DIDS (4,4'-dithiocyano-2,2'-dihydrostilbene disulfonic acid) at 0-4C leads to preferential labeling of the glycocalyx. 3H2DIDS-labeled cells incubated at 37C for 1 hr spontaneously released 3.5% of the total cell protein and 47 of the total 3H2DIDS. Repeated washings and resuspensions of 3H2DIDS-labeled cells with cold phosphate buffered saline also released protein and 3H2DIDS-labeled material. The amount released with each wash was essentially constant consisting of 0.45% of the total protein. The amount of H2DIDS-labeled material (cpm/ml glycocalyx) decreased with each wash. When separated by

SDS-PAGE these components were identical to those isolated from cells incubated at 37C. The glycocalyx removed as a result of repeated washings contained no cytochrome oxidase, essentially no diaphorase and low levels of lactic dehydrogenase. It is concluded that the material shed by Ehrlich cells is of surface origin and very labile. (no Refs)

65. PROTEINASE-MEDIATED LOSS OF ADHESION OF HUMAN MELANOMA CELLS FOLLOWING BINDING OF ANTI-MELANOMA ANTIBODY (MEETING ABSTRACT).

Singer KH, Stuhlmiller GM, Hashimoto K, Lazarus GS
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Fed Proc; 41(3):555 1982

Alteration of adhesive properties of malignant cells might greatly influence the metastatic potential of tumors. We have studied the effects of antibody binding on the adhesive properties of melanoma cells. Monolayer cultures of melanoma cells were incubated with an IgG fraction from unimmunized monkey (NM) serum or from a hyperimmune monkey anti-human melanoma (a-mel) serum. Following treatment with 1 mg/ml NM IgG, 15-30% of cells detached from the culture dish. In contrast, 50-80% of cells detached following treatment with 1 mg/ml a-mel IgG. There was no cytotoxicity. Antibody induced cell detachment was partially inhibited by DFP and completely inhibited by lima bean trypsin inhibitor. Thiol and carboxyl proteinase inhibitors did not block antibody induced cell detachment. An IgG dose dependent increase in DFP (diisopropylfluorophosphate) inhibitable caseinolytic activity was demonstrated in the culture medium of melanoma cells incubated with a-mel IgG, but not in those incubated with NM IgG. Medium collected from antibody treated cell cultures and incubated with Protein A Sepharose to remove IgG caused detachment of cells in fresh cultures. Preincubation of this conditioned medium with DFP abolished the medium's ability to mediate loss of cellular adhesion. These data suggest that incubation of melanoma cells with immune IgG results in release of a serine proteinase which mediates loss of cellular adhesion. (no Refs)

66. CULTURE OF NORMAL AND MALIGNANT MAMMARY EPITHELIUM ON MAMMARY GLAND EXTRACELLULAR MATRIX (MEETING ABSTRACT).

Wicha MS, Lowrie GB, Mahn T
University of Michigan, Ann Arbor, MI, 48109
Proc Am Assoc Cancer Res; 23:915 1982

We have developed an in vitro system for the primary culture of normal and malignant mammary epithelium on a complex extracellular matrix derived from rat mammary glands. This matrix promotes cell attachment, growth, differentiation and long term survival of these cells. Extracellular matrix was prepared from midpregnant rat mammary glands by sequential extraction with water, 1m NaCl, butanol: diisopropyl ether and DNase and RNase. The resultant material contains the basement membrane components type IV collagen and laminin as well as fibronectin as determined by immunofluorescence. Primary cultures of normal rat mammary epithelium plated on mammary gland extracellular matrix (MGEM) produce 50 times the alpha-lactalbumin of those on tissue culture plastic or collagen coated dishes as determined by radioimmunoassay. Unlike cultures on floating collagen gels, those on MGEM continue to secrete the milk sugar lactose for over 30 days. Neither extracellular matrix prepared from rat liver nor the purified basement membrane components, type IV collagen or laminin are able to substitute for MGEM in the support of mammary differentiation. Cells on the surface of MGEM continue to synthesize DNA as determined by H3-thymidine incorporation and autoradiography. This continues until the matrix is covered by a confluent cell layer. In contrast, there is little DNA synthesis in cells on floating collagen gels. We have found that MGEM also facilitates the culture of malignant human mammary cells. Primary cultures obtained from human breast cancer biopsy specimens or pleural effusions attach to and proliferate on MGEM more readily than tissue culture plastic or collagen coated dishes. This system provides a new tool for the culture of normal and malignant mammary epithelial cells, and support the concept that extracellular matrix components are important in mammary growth and differentiation. (no Refs)

67. ANTIGENS OF THE BASEMENT MEMBRANE AND THE PERITUMORAL STROMA IN HUMAN COLONIC ADENOCARCINOMAS: AN IMMUNOFLUORESCENCE STUDY.

Burtin P, Chavanel G, Foidart JM, Martin E
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Int J Cancer; 30(1):13-20 1982

Twenty colonic adenocarcinomas were studied by immunofluorescence with antisera against components of the basement membrane: Type IV collagen, laminin and heparan sulfate-rich proteoglycan, as well as antisera against antigens of the connective tissue: Type-III collagen, fibronectin and hyaluronectin. Marked alterations of the basement membranes were consistently observed on staining with each one of the first three antisera. In contrast, staining of the normal components of connective tissue was in most cases as intense as in normal colonic mucosa. Hyaluronectin, a marker of peritumoral stroma, was found to be present in 12 out of 15 tumors studied. (Author abstract) (22 Refs)

OTHER RELATED STUDIES

68. CANCER-CELL ORGANELLES.

Reid E, Cook GM, Morre DJ
Wolfson Bioanalytical Unit, Univ. Surrey, Robens Inst. Industrial and Environmental Health and Safety, Surrey, England
Methodol Surv Biochem 11: 415 pp., 1982.

Methods for obtaining reputable cell populations as organelle sources are discussed. Starting materials range from solid tumors to non-tissue cells with reference to rat hepatomas and human tumors and technics such as flow cytometry. Topics presented in relation to intact cells taken directly from in vivo sources include identification and separation of malignant cells from normal cells in tumors, separation of tumor and host cell populations from human neoplasms, identification of cancer cells by their surface antigens, non-enzymatic separation of tumor cells from Lewis lung carcinoma, and separation and short-term culture of different cell types from a transplanted hepatoma. Growth and characterization of normal and leukemic cell populations in vitro and demonstration of malignant transformation of cells in vitro are discussed. Considerations related to cancer cells and to RNA include phenotypic markers of hepatic 'preneoplasia' and neoplasia in the rat, and problems in separating membranous cell components from transplanted hepatomas. Isolation and preparation of nuclei, mitochondria, lysosomes, peroxisomes, endomembranes, plasma

membranes, and cell surfaces from neoplastic cells is discussed. (920 Refs)

69. THE TRANSFORMED CELL.

Cameron IL, Pool TB
Dept. Anatomy, Univ. Texas Health Science Center at San Antonio, San Antonio, TX, 78284
The Transformed Cell. New York, Academic Press, 435 pp., 1981.

Differences between normal and neoplastic transformed cells are described. Topics discussed include a brief description of terminology and basic concepts; background material related to evidence for and against the possible correlation of in vivo tumorigenicity to in vitro changes in the cytoskeletal system, anchorage-dependent growth, plasminogen activator production, agglutinability by lectins, and cell surface and plasma membrane properties; general aspects of regulation of cell proliferation and the relationships between ion movement and energy metabolism in normal and transformed cells; viral transformation of cells; and selected cellular properties, including cyclic nucleotides, polyamine metabolism, mobility of cellular water, cell viscosity, intracellular pH, and element concentration. (1490 Refs)

70. THE ROLE OF VIRAL AND ONCOFETAL ANTIGENS IN MURINE LYMPHOSARCOMA METASTASIS TO SPECIFIC SITES (MEETING ABSTRACT).

Nicolson GL, Reading CL, Mascali JJ, McGuire EJ
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Proc Am Assoc Cancer Res; 23:181 1982

Metastatic variants of murine RAW117 lymphosarcoma were established by sequential selection in vivo for enhanced liver or lung colonization or in vitro for decreased binding to the immobilized-lectins Con A, WGA, RCA (concanavalin A, wheat germ agglutinin, red cell agglutinin) and PNA. The heterogeneity and stability of the selected sublines were examined using cloning techniques. Cell surface analysis of sublines and clones indicated that loss of cell surface viral envelope glycoprotein gp70 correlated well with metastatic properties suggesting that host response may be involved. A variety of in vivo experiments indicated that host macrophages may suppress high gp70 containing clones but allow growth of low gp70 containing clones. To determine the role of cell surface oncofetal antigens in organ colonization we tested the effects of antibodies made against fetal liver cell adhesion components. Liver specific colonization was blocked by pretreatment with Fab' or F(ab')2 antibodies made against the embryonic liver cells. The liver colonizing RAW117 sublines appear to have higher levels of the oncofetal antigen(s) compared to parental or lung colonizing tumor cells. (2 Refs)

71. THE EFFECT OF MEMBRANE FATTY ACID MODIFICATIONS ON DEPOLARIZATION-DEPENDENT EXOCYTOSIS IN THE CLONAL PHEOCHROMOCYTOMA CELL LINE, PC-12.

Williams TP
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Diss Abstr Int [B]; 42(9):3655-B 1982

The effect of changes in membrane lipid composition on the exocytotic release process was studied in the PC12 cells. None of the fatty acid modifications affected K+-stimulated release of 3H-norepinephrine. This observation indicated that the individual steps of the secretion process, including and subsequent to activation of membrane potential-dependent Ca++ channels were unaffected by the fatty acid changes. In contrast, both carbamylcholine and veratridine-stimulated exocytosis were diminished in cells enriched with unsaturated fatty acids. The diminished output of 3H-norepinephrine was observed at all concentrations of carbamylcholine and veratridine tested, with no change in the relative concentration-dependence. These results may have been due to a decrease in the number of receptors or ion channels, or due to a decrease in the ability of these membrane proteins to initiate ion fluxes. The decrease in carbamylcholine-stimulated release did not correlate with the simple presence of the fatty acids, but paralleled closely the time and concentration-dependent changes in phospholipid fatty acid composition. However, when oleate and arachidonate were simultaneously added to the culture medium, the inhibitory effects on carbamylcholine-stimulated release were additive, whereas the changes in phospholipid fatty acid composition were antagonistic. Thus, exposure of the PC12 cells to unsaturated fatty acids causes specific decreases in the activities of at least two stimulus/secretion processes. In addition, the decrease in receptor-mediated (carbamylcholine-stimulated) release was related to the concentration and time of exposure to the fatty acids, and it was reversible. However, the mechanism by which exogenous fatty acids produce these effects on release cannot be readily deduced from simple analysis of bulk membrane lipid composition. (no Refs)

72. EFFECT OF CELLULAR FATTY ACID ALTERATION ON HYPERTHERMIC SENSITIVITY IN CULTURED L1210 MURINE LEUKEMIA CELLS.

Guffy MM, Rosenberger JA, Simon I, Burns CP
Dept. Medicine, Univ. Hosp., Iowa City, IA, 52242
Cancer Res; 42(9):3625-3630 1982

We investigated the effect of fatty acid alteration on the thermosensitivity of a mammalian tumor cell, the L1210 lymphoblastic leukemia. Cells growing in Roswell Park Memorial Institute Medium 1640 with 5% fetal calf serum were modified with respect to their fatty acid composition by supplementing the media with either 40 uM docosahexaenoic acid (22:6) or oleic acid (18:1). A soft-agar clonogenic assay was used to assess viability following hyperthermic exposure. Cells grown in 22:6-supplemented media were more sensitive (Do 18.7 +-0.4 min), and cells grown in 18:1-supplemented media were less sensitive (Do 46.2 +-1.6 min) to a 42 C exposure than were cells grown in unsupplemented media (Do 27.5 +-1.5 min) (p less than 0.001 for all comparisons). These contrasting effects on thermosensitivity increased as the concentration of supplemental fatty acid increased from 0 to 40 uM and were apparent at all temperatures from 41 to 44 C. When compared to cells grown in unsupplemented media, those grown in 22:6-supplemented media contained 300% more polyenoic and 70% less monoenoic fatty acid in their cell phospholipids, while those grown in 18:1-supplemented media contained 37% less polyenoic and 17% more monoenoic fatty acid. Similar changes were noted in neutral lipid fatty acid composition, while no changes were noted in cellular cholesterol or phospholipid content. Electron spin resonance studies suggested a significant increase in membrane fluidity when cells were grown in 22:6-supplemented media and a decrease when cells were grown in 18:1-supplemented media. These data demonstrate that modification of the cellular fatty acid composition of a mammalian tumor cell dramatically affects its thermosensitivity. (Author abstract) (48 Refs)

73. EFFECT OF FATTY ACID MODIFICATION OF CULTURED HEPATOMA CELLS ON SUSCEPTIBILITY TO NATURAL KILLER CELLS.

Yoo TJ, Kuo CY, Spector AA, Denning GM, Floyd R, Whiteaker S, Kim H, Kim J, Abbas M, Budd TW
Dept. Medicine, Univ. Tennessee Center for Health Sciences, Veterans Admin. Medical Center, Memphis, TN, 38163
Cancer Res; 42(9):3596-3600 1982

Rat hepatoma (Morris 7777) cells modified with either oleic or linoleic acid exhibited greater susceptibility to normal spleen cell-mediated lysis in a 16-hr 51Cr release assay. At effector:target cell ratios of 300:1, the specific lysis of fatty acid-enriched target cells (cultured for 2 days in fatty acid-supplemented medium) by the normal rat spleen cells was 60% higher than the untreated target cells (p less than 0.01). Prolonging the culture in fatty acid-supplemented medium up to 6 days produced similar effects. Analysis of the fatty acid composition of cellular lipids revealed that an elevation of oleic or linoleic acid was the only significant alteration in the hepatoma cells grown in the oleic or linoleic acid-supplemented medium, respectively. The percentage of the acids was increased in the total cellular phospholipids, the choline, ethanolamine, serine, and inositol phosphoglyceride fractions, and the neutral lipids. In conclusion, we suggest that the elevation of oleic acid and linoleic acid contents in the membranes of the fatty acid-modified hepatoma cells may contribute to the increased susceptibility of these cells to natural killer cell-mediated cytotoxicity. (Author abstract) (33 Refs)

74. CYTOTOXICITY OF NATURAL KILLER CELLS. CORRELATION WITH EMPERIPOLESIS AND SURFACE ENZYMES.

Burns ER, Zucker-Franklin D, Valentine F
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Lab Invest; 47(1):99-107 1982

Cell-mediated cytotoxicity involving natural killer cells requires contact between effector and target cells for effective cytolysis. Ultrastructural studies of biopsies of primary human malignant melanoma showed mononuclear leukocytes to be located in close proximity to tumor cells and, on occasion, within the confines of the melanoma cell itself. This phenomenon, called emperipolesis, was examined in vitro to determine whether the same population of cells that exhibits emperipolesis is responsible for cytotoxicity. Since natural killer cells have been identified morphologically and functionally as large granular lymphocytes with surface receptors for the Fc portion of immunoglobulin (FcR+ cells), lymphocytes were depleted of FcR+ cells, and their cytotoxicity and ability to emperipolese were measured. Both of these properties were markedly diminished (88 and 85%, respectively). Systematic comparison of emperipolesis and cytotoxicity from donors known to exhibit either high or low lymphocyte cytotoxicity showed perfect concordance. Ultrastructural analysis of in vitro emperipolesis revealed the emperipolesing lymphocytes to be FcR+ cells establishing identity with the large granular cells known to mediate cytotoxicity. The morphologic marker found in both FcR+ cells, purified by rosetting techniques, and in emperipolesed lymphocytes consisted of cytoplasmic parallel tubular arrays. Further studies designed to elucidate a mechanism for cytotoxicity and emperipolesis implicated cell surface proteases as mediators of these activities. Competitive inhibition of surface proteases with artificial and natural inhibitors markedly reduced both cytotoxicity and emperipolesis. Therefore, it is likely that lymphocytes that are FcR+ participate in cell-mediated cytotoxicity through mechanisms involving cell contact and enzyme-initiated damage of target cells. Emperipolesis represents one type of effector-target cell contact leading to cytotoxicity. (Author abstract) (43 Refs)

75. GANGLIOSIDE GM1 SENSITIZES TUMOR CELLS TO GROWTH INHIBITORY GLYCOPEPTIDES.

Kinders RJ, Rintoul DA, Johnson TC
Div. Biology, Kansas State Univ., Manhattan, KS, 66506
Biochem Biophys Res Commun; 107(2):663-669 1982

A glycopeptide prepared from the surfaces of cerebral cortex cells of Swiss albino mice by mild proteolysis inhibits cellular protein synthesis in normal but not transformed cells. Treatment of C3H mouse fibrosarcoma 1316 cells with the glycopeptides resulted in only a limited degree of inhibition of cell protein synthesis compared to polyoma virus-transformed baby hamster kidney cells. The previously described possible role of ganglioside GM1 as a receptor for a number of glycoproteins with physiological activity suggested that the insensitivity of 1316 cells to the glycopeptide growth inhibitor (GGI) might be due to insufficient levels of GM1 in the cell surface membrane. When 1316 cells were incubated with GM1 under conditions in which the ganglioside is incorporated into the cells, the cells became sensitive to GGI. In parallel studies with ceramide, there was no change in 1316 sensitivity. When 1316 cells were incubated with GM1 for 18 hr, they appeared to metabolize GM1 to simpler gangliosides and lost their sensitivity to the glycopeptide inhibitor. (17 Refs)

76. MEMBRANE LIPID MODIFICATION OF CHRONIC OPIATE EFFECTS IN NEUROBLASTOMA X GLIOMA NG108-15 HYBRID CELLS (MEETING ABSTRACT).

Griffin MT, Law PY, Loh HH
Department of Pharmacology, University of California, San Francisco, CA, 94143
Fed Proc; 41(3):1220 1982

Neuroblastoma x glioma NG108-15 hybrid cells chronically exposed to opiates exhibit elevated adenylate cyclase activity upon removal of the opiate agonist. This increase in adenylate cyclase activity, described as a 'dependent' response, has been shown to require a lipid component. In order to more fully characterize this lipid requirement, NG108-15 cells were grown in a serum free, chemically defined medium according to the method of Sato and the involvement of specific lipid components were studied through the selective hydrolysis of the membrane lipids by phospholipase A2 and C. Cells grown for up to 18 days in serum free medium displayed little or no change in opiate acute or chronic action which suggests that serum lipids or essential fatty acids are not required for opiate receptor regulation of adenylate cyclase. Similarly, hydrolysis of the beta 2 fatty acid moieties by phospholipase A2 had no effect on opiate action. However, modification of the polar head groups of the membrane lipids by phospholipase C (PLC) attenuated the acute opiate inhibition of adenylate cyclase by 40% and attenuated the chronic dependent response by 80% without significantly affecting specific opiate binding. Specificity of the polar head groups is evident from the fact that PLC from Bacillus Cereus does not attenuate opiate responses while PLC from Clostridium Welchii does. (no Refs)

77. IN VITRO CHARACTERIZATION OF THE ADJUVANT ACTIVITY OF LIPOSOMES CONTAINING HUMAN TUMOR ANTIGENS (MEETING ABSTRACT).

Raphael L, Liu JS, Tom BH
University of Texas Medical School, Houston, TX, 77030
Fed Proc; 41(3):416 1982

Liposomes prepared with LS174T human colon tumor cell membranes stimulated the primary in vitro generation of cytotoxic BALB/c splenocytes (SPC). The kinetics of the induction of SPC was similar for both whole LS174T cells and liposome antigen (MLV-Ag). Specificity of the primed SPC was demonstrated on a variety of human tumor cells. Cold target inhibition results revealed 72% of the cytotoxic activity directed at tumor and not HLA antigens. Elimination of Thy 1.2+ cells abolished the lytic activity of the immune cells indicating T cell involvement. Addition of Thymocyte-conditioned media improved the ability of antigens to elicit effector cells. The adjuvant effect of MLV-Ag is mediated primarily by tumor antigens exposed on the outer surface of liposomes. Trypsinization of the MLV-Ag which eliminated 96% of the surface protein reduced the ability of the MLV-Ag to induce cytotoxic SPC. Dose response of MLV-Ag showed that while 10ug was the threshold, 100ug protein induced maximal responses. Since only 50% of the Ag is exposed on the MLV-Ag surface a critical Ag density of 50ug is necessary for the induction of cell-mediated immunity. Parallel studies with breast tumor Ag have now been shown to generate specific cytotoxic cells. Ag have now been shown to generate specific cytotoxic cells. Thus, MLV-Ag may provide a means to investigate cell-Ag conditions necessary for inducing cytotoxic T cells, as well as a potential system for selecting desired immune responses. (no Refs)

78. MICROINJECTION OF INTERFERON AND 2',5'-OLIGOADENYLATE INTO MOUSE L CELLS AND THEIR EFFECTS ON VIRUS GROWTH.

Higashi Y, Sokawa Y
Dept. Cellular and Molecular Virology, Inst. for Virus Res., Kyoto Univ., Sakyo-ku, Kyoto,, Kyoto 606, Japan
J Biochem (Tokyo); 91(6):2021-2028 1982

By means of a new type of microinjection apparatus, which has a micropipette located in a hole through the optical axis of the condenser lens, we injected interferon (IFN) or 2',5'-oligoadenylate (2-5A) into mouse L cells, and observed their antiviral effects on the multiplication of vesicular stomatitis virus (VSV). After injection, cells were infected with VSV, and labeled with [3H]uridine in the presence of actinomycin D. The proportion of cells infected with VSV which carried radioactive virus-RNA was determined by autoradiography. IFN introduced directly into L cells had no effect on the virus growth. This result supports the idea the IFN molecules exert their effect from outside the cell membrane without penetrating into the cytoplasm. 2-5A, on the other hand, was able to inhibit the growth of VSV effectively when injected into L cells. The antiviral effect was dependent on the dose of 2-5A injected, and moreover the effect was transient, since it disappeared completely after 24-h incubation. (Author abstract) (28 Refs)

79. IDENTIFICATION OF THE PLATELET ALLOANTIGEN (PI[A1]) IN CIRCULATING IMMUNE COMPLEXES OF NORMAL HUMAN SERA.

Wautier JL, Boizard B, Doan R, Caen JP, Hartmann L
Dept. Immunohematology, Hopital Lariboisiere, 2 rue A. Pare, F-75475 Paris, Cedex 10, France
Cancer Immunol Immunother; 13(1):44-47 1982

Cell membrane fragments associated with polyethylene glycol (PEG) precipitates were examined to find out whether the fragments represent remnants of platelet membranes. The PI[A1] alloantigen present on platelets was used as antigenic marker for identification of platelet fragments. Unabsorbed anti-PI[A1] serum gave positive results at a dilution of 1/16-1/8. The inhibition of the reaction of anti-PI[A1] serum with PI[A1]-positive platelets was proportional to the amount of circulating immune complexes (CIC) incubated with the anti-PI[A1] before the immunofluorescence test. Anti-PI[A1] serum induced 89% of the maximum platelet lysis. Prior incubation of antisera with CIC inhibited lysis which was proportional to the amount of CIC added: there was 100% inhibition with 940 ug and 50% inhibition with 470 ug. Anti-PI[A1] serum was used undiluted or diluted in parallel experiments. A one-half dilution of the serum induced 68% of maximum lysis. Complete inhibition was observed with 470 ug CIC when the anti-PI[A1] was used at this dilution. The percent inhibition of chromium lysis by CIC was not significantly modified when the platelets were treated with bromelain. Results of the inhibition of immunofluorescence and of the inhibition of chromium lysis assays indicated that material reacting with anti-PI[A1] antibodies is present in 3.5% PEG precipitates of normal human sera and that some of the cell fragments in CIC could represent platelet membrane. (19 Refs)

80. HYBRIDOMAS THAT SECRETE MONOCLONAL ANTIBODIES AGAINST HUMAN NORMAL AND CANCER CELLS (MEETING ABSTRACT).

Lee LS
General Electric Research and Development Center, Schenectady, NY, 12301
Hybridoma; 1(2):225 1982

A human lung-carcinoma-derived cell line was used to produce antibodies against tumor-associated antigens. About 10(7) cells which were frozen and thawed once, were injected ip into a female Balb/C mouse once per week for 4 weeks. The spleen of the mouse was removed and the lymphocytes were purified and fused with the mouse myeloma cell line SP2/0. Optimal hybridization was obtained when the hybridization cultures were exposed to Carbowax 3350 for a total of 10 min. A solid phase enzyme-linked immunoassay was employed to screen 67 hybridoma supernatants for reactivity with normal and tumor cells grown in monolayers. The results suggest that normal membrane antigens may become nondetectable on cancer cells because they are misplaced inside the cells. Similarly, tumor antigens may arise from the dislocation of normal intracellular antigens onto the cell surface. Cell surface antigen loss, as well as neoantigen appearance, both detectable by monoclonal antibodies, may both be useful in the immunodiagnosis of cancer. (no Refs)

81. THE FORMATION OF LOW RESISTANCE JUNCTIONS BETWEEN REAGGREGATING NOVIKOFF HEPATOMA CELLS.

Kam EY
University of Minnesota, Minneapolis, MN
Diss Abstr Int [B]; 42(6):2183-B 1981

The effects of temperature, pH/CO2, recovery from EDTA dissociation and trypsin treatment on the formation of low resistance junctions between EDTA-dissociated, then reaggregated Novikoff hepatoma cells were studied with the standard electrophysiological technique using three glass electrodes. The percentage of pairs that were electrically coupled (coupling percentage) and the junctional conductance obtained from studying reaggregated pairs were used as quantitative descriptions of formation. Coupling percentage decreased progressively from 30C (93%), to 10C (27%) in the presence of 5% CO2. Not much change was found in coupling percentage when the temperature was raised to 42C. Junctional conductance increased progressively from 0.17 +/-0.14 (SEM) micromho at 10C to 1.46 +/-0.45 micromho at 42C. Incubating cells in ambient level of CO2 and 37 degrees reduced the coupling percentage from 89% (with 5% CO(2)) to 33%, while the junctional conductance was also reduced from 1.08 +/-0.24 micromho to 0.19 +/-0.08 micromho. In contrast, incubating cells at ambient CO2 tension and room temperature (c 21) seemed to inhibit formation of junctions. Using HEPES as buffer in the incubating medium reduced the formation of junctions independent of the level of pH (from 6.8 to 8.0). However, when bicarbonate was used as buffer, cells incubated in medium titrated to pH equals 7.4 initially had a higher coupling percentage (100%) than those incubated in medium titrated to pH equals 8.4 (88%) initially. The junctional conductance also decreased from 0.43 +/-0.01 micromho (pH equals 7.4) to 0.15 +/-0.06 micromho (pH equals 8.4). Recovery from EDTA dissociation seemed to have only minimal effect on formation of junctions. On the other hand, cells treated with increasing concentration of trypsin showed a decrease in coupling percentage as well as junctional conductance in the lower range (from 0 mg/ml to 0.005 mg/ml), but a partial restoration in the higher range (from 0.005 mg/ml to 0.015 mg/ml). (no Refs)

82. A METHOD TO QUANTIFY THE POTENCY OF PHOTOSENSITIZERS THAT MODIFY CELL MEMBRANES.

Pooler JP, Valenzeno DP
Dept. Physiology, Emory Univ., Atlanta, GA, 30322
JNCI; 69(1):211-215 1982

The numerical assessment of sensitizing potency of different species of photosensitizer molecules has historically been impractical because of the difficulty of measuring all of the factors that must be taken into account. This paper describes a new method for quantifying relative sensitizer potency that obviates most of the past difficulties. The physical data needed to assess photosensitizing potency are the absorption spectrum of a sample of medium containing sensitizer, the illumination spectrum impinging on the preparation, the duration of illumination, and the amount of light-induced modification. The measurement of these quantities is relatively straightforward, but it is easy to overlook a number of artifacts that can distort the values for potency. Avoiding error requires careful attention to the optical properties of the system, particularly in the light path through the medium containing sensitizer. (Author abstract) (11 Refs)

83. MOLECULAR ACTIONS AND TARGETS FOR CANCER CHEMOTHERAPEUTIC AGENTS.

Sartorelli AC, Lazo JS, Bertino JR
Dept. Pharmacology, Comprehensive Cancer Center, Yale Univ. Sch. Medicine, New Haven, CT, 06510
Bristol-Myers Cancer Symp Ser 2: 598 pp., 1981.

New approaches to anticancer treatment, important aspects of the molecular actions of anticancer drugs, and possible new biochemical targets for therapeutic agents are discussed. Topics discussed include alkylating agents (molecular mechanisms of cross-linking by alkylating agents and Pt complexes, mechanism of action of antitumor Pt coordination compounds, macromolecular targets and metabolic properties of alkylating drugs, chemotherapeutic approaches to cell populations of tumors, suicide tumor inactivators); antibiotics (DNA dynamics and drug intercalation, molecular pharmacology of anthracyclines, protein antibiotics as DNA-damaging agents, action of bleomycin on DNA); nucleoside analog antimetabolites (transport by animal cells, purine nucleoside phosphorylase and 5'-methylthioadenosine phosphorylase in chemotherapy, effects of 5'thioadenosine nucleosides, locus of action of fluorouracil, inhibition of thymidylate synthetase); folate analog antimetabolites (binding of ligands to dihydrofolate reductase, transport of folate compounds in L1210 cells, membrane transport and molecular basis for antitumor action of folate analogs, cellular mechanisms of resistance to methotrexate); radiation sensitizers (hypoxia-dependent radiation sensitizers and chemotherapeutic agents, mechanistic and pharmacologic considerations); membrane targets (plasma membranes; altered plasma membrane glycoconjugates of Chinese hamster cells with acquired resistance to actinomycin D, daunorubicin, and vincristine; tubulin); and angiogenesis, metastasis, and anticarcinogenesis differentiation (angiogenesis inhibitors, effects of beta-all-trans-retinoic acid on growth and implantation properties of metastatic B16 melanoma cell lines, retinoids and suppression of polypeptide transforming factors, new hematopoietic cell systems, induction of normal differentiation in malignant cells as an approach to cancer therapy). (1112 Refs)

84. MECHANISMS OF ANTHRACYCLINE RESISTANCE AND CYTOTOXICITY IN CULTURED SARCOMA 180 CELL LINES.

Siegfried JM
Yale University, New Haven, CT
Diss Abstr Int [B]; 42(6):2329-B 1982

Sublines of Sarcoma 180 (S180) cells of varying resistance to the chemotherapeutic agent adriamycin (doxorubicin) (ADR) have been selected in culture. The degree of resistance of these sublines, ranged from 6-to 125-fold above that of the parent S180 cells. Increasing resistance to ADR was accompanied by increasing cross-resistance to the anthracyclines daunomycin (DNR), N-trifluoroacetyl-adriamycin-14-valerate, and marcellomycin. These drug-resistance sublines have been used in comparison to the parent S180 cells to examine factors which may be important in the cytotoxic action of the anthracyclines. Decreased drug permeability was found to be of minimal importance in the expression of resistance. Inhibition of nucleic acid synthesis after exposure to ADR was not predictive of cell survival. An oxygen atmosphere was not required for the cytotoxicity of ADR and DNR in either anthracyclines or resistant cells. Futhermore, the cytotoxicity of the anthracyclines was specifically increased under a hypoxic atmosphere, although drug levels and metabolism were equivalent under conditions of normal aeration and hypoxia. Evidence was found, however, of differences in the membrane properties of cells ex-

posed to the two conditions of aeration. The fluidity of cellular membranes, reversibly decreased after exposure to chronic hypoxia. The increased susceptibility to ADR observed after hypoxic stress was also reversible upon reoxygenation. Progressive changes in membrane fluidity were also correlated with increasing resistance to the anthracyclines. These results suggest that inhibition of nucleic acid synthesis and generation of superoxide radical may not be involved in the cytotoxicity of ADR and DNR to S180 cells. Activation of anthracyclines via one or two electron transfer probably is not responsible for cytotoxicity. Changes at the level of the plasma membrane, which are dissociable from changes in drug permeability, may be important in susceptibility to the anthracyclines. (no Refs)

85. ACTIONS OF CIS-DIAMMINEDICHLOROPLATINUM ON CELL SURFACE NUCLEIC ACIDS IN CANCER CELLS AS DETERMINED BY CELL ELECTROPHORESIS TECHNIQUES.

Juckett DA, Rosenberg B
(c/o Rosenberg) Dept. Biophysics, Michigan State Univ., East Lansing, MI, 48824
Cancer Res; 42(9):3565-3573 1982

Whole-cell electrophoresis determinations were made on a variety of tumor cell suspensions in order to examine the charged groups on the cell surfaces. We report here evidence which indicates the presence of nucleic acids on the surfaces of the tumor cells. Cells of the ascitic and solid forms of the Sarcoma 180 mouse tumor, grown in ICR mice, showed a decrease of 10 to 20% in electrophoretic mobility when incubated with nucleases. One variant, S-180, exhibited sensitivity to RNase only; another variant was sensitive to both RNase and DNase, while different tumor lines were shown to be sensitive to DNase only. Treatment of tumor-bearing animals with a therapeutic dose of cisplatin resulted in a loss of tumor cell mobility identical to that produced by the nuclease incubations of the control cells. Incubation of the cisplatin-treated tumor cells with nucleases produced no change in mobility. The isoelectric points of these cells are consistent with the loss of a group with a low pK value in both cisplatin-treated cells and nuclease-incubated cells. Using the S-180 tumor sensitive to both RNase and DNase, the rates of the two enzymes were additive, but the mobility decreased to the same level regardless of whether the enzymes were used alone or together. This suggests that both enzymes act on the same site. RNase and DNase immobilized on agarose beads were capable of lowering the mobility of the cells upon incubation, confirming the surface location of these nucleic acid residues. S-180 tumor cells were also placed in a tissue culture medium at 37 C for up to 22 hr and were treated in vitro with cisplatin and other metabolic inhibitors. Nucleic acid or protein synthesis inhibitors produced a loss of the cell surface nucleic acids. In another in vitro experiment, the nucleic acids were first removed by nucleases, and, when the cells were incubated at 37 C, the nucleic acids reappeared. Disappearance with the inhibitors or reappearance after digestion exhibited a half-time of about 2 hr. Surface nucleic acids were detected by this electrophoresis technique on all of several types of tumor cells but not on any normal cells examined. (18 Refs)

86. THE ANTICANCER AGENT ADRIAMYCIN CAN BE ACTIVELY CYTOTOXIC WITHOUT ENTERING CELLS.

Tritton TR, Yee G
Dept. Pharmacology, Yale Univ. Sch. Medicine, New Haven, CT, 06510
Science; 217(4556):248-250 1982

The antineoplastic agent adriamycin was coupled to an insoluble agarose support. This material was actively cytotoxic to L1210 cells in culture under conditions in which no free adriamycin could enter the cell. It is concluded that an agent whose principal target was previously thought to be DNA can exert its cytotoxic action solely by interaction at the cell surface. (Author abstract) (16 Refs)

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